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Alaska Oil Spill Bioremediation Project

Science Advisory Board Draft Report

Sections 1 through 6

ALASKA OIL SPILL BIOREMEDIATION PROJECT

SCIENCE ADVISORY BOARD DRAFT REPORT

SECTIONS 1 THROUGH 6

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ABSTRACT

The Alaska Oil Spill Bioremediation Project was initiated to demonstrate the feasibility of oil bioremediation as a secondary cleanup tool on selected beaches in Prince William Sound, and to further the understanding of the microbial ecology of oil biodegradation on shorelines. It was shown that the addition of oleophilic slow-release/granular and nutrient solution fertilizers to oil-contaminated beaches in Prince William Sound increased oil biodegradation rates greater than four-fold over removal rates on untreated oiled beaches. The application of fertilizer solutions proved to be the most efficient system for exposing oil-degrading microorganisms to nutrients. Data on the rate and extent of microbial degradation of oil was crucial to the acceptance of bioremediation as a cleanup technology. This enhanced biodegradation was evidenced by changes in several constituent hydrocarbon groups resulting in the disappearance of oil residues. Supporting studies demonstrated that bioremediation of oil is a reasonable and environmentally sound secondary cleanup procedure. It appears to work in both surface and subsurface beach material. Although there was an overall lack of general oil biodegradation at Disk Island, studies during the summer of 1990 at Elrington Island showed that a pulse application of nutrients provides sustained accelerated biodegradation of oil over a three to four week period. This pulse application phenomenon has significant potential for addressing future oil spills since it is as effective as a continuous long-term application. In addition, the use of sampling baskets containing homogenized beach material was a reliable method to supplant direct sampling of beach material.

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EXECUTIVE SUMMARY

The feasibility of using bioremediation as cleanup tool for oil contaminated beaches in Prince William Sound, Alaska, has been verified by a series of field and laboratory studies conducted during 1989 and 1990. Initial field studies at Snug Harbor during the first summer involved the application of two different types of fertilizer formulations (oleophilic and slow-release) directly to the beach surface. The results from this study provided sufficient information to allow Exxon to consider bioremediation on a large scale during the month of August 1989 to assist in the overall oil cleanup program. Approximately 70 miles of the Prince William Sound beaches were subsequently treated with a combination of oleophilic and slow-release fertilizers. A second field study at Passage Cove during the first summer was used to verify the effectiveness and safety of this large-scale application.

Following laboratory research during the winter of 1989/1990, activities were initiated for a second summer to optimize fertilizer application. Field studies were conducted at sites on Elrington Island and Disk Island. The research results demonstrated the effectiveness of pulsed fertilizer application and established new methods for assessing the effectiveness of bioremediation. Enhancement of oil biodegradation was greater during the summer of 1990 than the previous summer.

Specific summaries and conclusions from the studies at each of the field sites is given below.

Snug Harbor

Two fertilizer formulations, an oleophilic fertilizer and fertilizer briquettes, were tested separately at Snug Harbor. Two types of contaminated beach material were also evaluated: cobblestone overlying mixed sand and gravel, and mixed sand and gravel alone.

a) Visual inspection of beaches treated with oleophilic fertilizer showed that oil was removed from the beach surface approximately 2 to 3 weeks after fertilizer application. The effect was most apparent on cobble beaches, where initially much of the surface oil was removed. No visible decreases in the oil occurred, however, on the beaches treated with the slow-release fertilizer briquettes or on the untreated control beaches. Disappearance of oil on oleophilic-treated plots continued over time, eventually leading to the disappearance of oil from most of the beach material surfaces.

b) No oil slicks or oily materials were observed in the seawater following application of the fertilizers, and no oil or petroleum hydrocarbons were detected in mussels contained in cages just offshore from the fertilizer-treated beaches. This suggested that removal of oil from the beaches did not appear to be a result of dispersing phenomena.

c) Analysis of oil extracted from all beach plots showed that the oleophilic fertilizer caused the greatest initial reduction in oil residues on the cobble surfaces, accompanied by substantial changes in oil composition. These results were significantly different when compared to the untreated control and the briquette fertilizer-treated plot. However, it is believed that the full extent of this change was initially masked through interferences · in the gas chromatographic analyses by components in the oleophilic fertilizer.

d) The greatest changes in oil composition were observed in samples from the briquette fertilizer-treated plots. However, changes were primarily observed during the first two weeks of the test in cobble plot samples, suggesting that the fertilizer-enhanced changes were short-lived. A more sustained but less extensive effect was seen in the mixed sand and gravel plots. These results lead to the conclusion that fertilizer briquettes, or a similar formulation that releases inorganic nitrogen and phosphorus, would likely affect changes in oil composition on both the cobble surface and within the mixed sand and gravel matrix.

e) All changes in oil composition were accompanied by large decreases in the nC18/phytane ratio. This represents a differential change in chemically similar hydrocarbons, and can only be attributed to biodegradation processes. Thus, fertilizer application appeared to enhance oil biodegradation.

f) Numbers of oil-degrading microorganisms did not appear to increase as a result of fertilizer application. However, large heterogeneity in the microbial population precluded observing statistical differences. This was further complicated by a high number of oil-degrading bacteria in the oiled beach material prior to fertilizer exposure (averaging 1 to 10% of the total bacterial population). The high numbers represented an enrichment of oil-degrading microorganisms of approximately 10^3 to 10^5 compared to beaches not exposed to oil. These results demonstrate that the beaches were well primed for bioremediation.

g) Extensive ecological monitoring studies indicated that the addition of fertilizer to oiled shorelines did not cause ecologically relevant increases in planktonic algae or bacteria, or any

measurable nutrient enrichment in adjacent embayments. These studies were supported by stable nitrogen isotope analyses of intertidal algae and heterotrophic organisms. Stable nitrogen isotope ratios demonstrated that when fertilizer was assimilated by algae on the beach, trophic structures were not disrupted. Finally, mutagenicity studies showed that mutagenic materials associated with Prudhoe Bay crude oil were lost over time from both treated and untreated control plots. In conjunction with chemical analysis, these studies demonstrated that decreases in mutagenicity were due to both fertilizer-enhanced biodegradation and other natural processes.

Passage Cove

Two fertilizer applications were also tested at Passage Cove. A combination of the oleophilic fertilizer and fertilizer granules (instead of briquettes) was applied to one beach, and a fertilizer solution was applied (via a sprinkler system each day at low tide) to another beach.

a) The visual reduction in oil due to application of the oleophilic/granular fertilizer combination was similar to Snug Harbor results. This visual reduction became apparent approximately two to three weeks following application of the fertilizers; the untreated control beach, on the other hand, essentially did not change visually. The effect was perhaps more dramatic in Passage Cove since oil from both the cobble surface and the subsurface mixed sand and gravel visually disappeared in a shorter timeframe. It is possible that when the beaches in Passage Cove were physically washed, oil was distributed over a large surface area, subsequently creating improved conditions for biodegradation of oil.

b) Application of fertilizer solutions from a sprinkler system also caused oil to visually disappear in approximately the same general timeframe as Snug Harbor results (3 to 4 weeks). This observation provided definitive proof that biodegradation (and not chemical washing) was likely responsible for the oil removal, since there is no other reasonable mechanism to explain this effect of nutrient addition to the oil. The application of fertilizer solutions, therefore, proved to be the most efficient system for exposing oil-degrading microorganisms to nutrients in a controlled and reproducible manner.

c) Application of the fertilizer solution produced a statistically significant enhancement of oil biodegradation relative to the untreated control beach. Rates of total oil residue loss were greater than four-fold faster than rates of removal on the untreated control beach. The loss

of oil residues was accompanied by extensive changes in oil composition. This included large decreases in the nC18/phytane ratio. Thus, enhanced biodegradation was probably responsible for changes in oil residue and composition. Results from the fertilizer solution treatment further support that oil biodegradation in Prince William Sound was limited by the availability of nutrients and not by the availability of the oil itself. In addition, reapplication of nutrients (the extreme in the case of the fertilizer solution) is probably important for sustaining enhanced biodegradation.

d) Application of the oleophilic/slow-release granular fertilizer combination also substantially enhanced oil biodegradation. At a slightly lower degree of statistical confidence (90% confidence level instead of 95%), this fertilizer combination produced a significant two-to three-fold enhancement in the removal of total oil residues relative to the untreated control beach. This was accompanied by an extensive change in the composition of the oil as well.

e) Mechanistically, there is no evidence to suggest that the application of the oleophilic/slow-release granular fertilizer combination worked differently than the application of the fertilizer solution; each process provided enough nutrients to the oil-degrading microbial populations to enhance biodegradation. Results from changes in oil composition during the initial two weeks following fertilizer application suggest that the oleophilic fertilizer uniquely caused simultaneous degradation of the higher and lower molecular weight hydrocarbons. Results from the untreated control beach and the fertilizer solution-treated beach showed that during the same time period, a more typical response was observed; that is, the lower molecular weight hydrocarbons degraded faster than the higher molecular weight hydrocarbons. It is also believed that the eventual greater response from the fertilizer solution application was due to higher nutrient concentrations sustained over a longer period. Reapplication of the oleophilic/slow-release granular fertilizer combination every three to four weeks might produce the same effect observed with the fertilizer solution application.

f) Further monitoring of the fertilizer-treated beaches through early summer 1990 revealed that even subsurface oil (to a depth of approximately 0.3 m) was virtually completely removed within approximately 10 months. However, significant, but patchy amounts of oil remained on the untreated control beach after this time period. This suggests that bioremediation greatly reduced beach cleanup time.

g) Due to high variability in the numbers of oil-degrading bacteria in each sample, it was not possible to show statistically significant increases in the oil-degrading microbial populations as a result of the fertilizer addition.

h) No widespread or persistent adverse ecological effects were observed from the monitoring program that was designed to measure toxic responses, eutrophication, and bioaccumulation of oil residues. Ammonia, the only component in the oleophilic fertilizer that was potentially toxic to indigenous species, never reached toxic concentrations outside the immediate zone of application (as inferred from the toxicity test results). Measurements of chlorophyll, primary productivity, and bacterial production indicated eutrophication did not occur. The absence of oil residues in caged mussels, held just offshore of the fertilizer-treated areas, supported the tenet that oil was not released from the beaches into the water column as a result of the fertilizer treatment.

Disk Island

A field study at Disk Island was conducted to estimate fertilizer dose response. Plots at Disk Island were dosed with different concentrations of slow-release granular fertilizer, to evaluate the effectiveness of concentrations above and below the recommended application rate. Effects on enhanced oil biogradation were measured. Special sampling baskets were developed for field sampling to ease the analytical burden.

a) Analysis of ammonia, nitrate and phosphate in interstitial beach water showed nutrient release was generally proportional to fertilizer application rate. However, nutrient release represented more of a pulsed high concentration during the first 2 to 3 days following application rather than a slow release over time. Stable nitrogen isotope analysis confirmed that the fertilizer was the source of NH_4^+ and NO_3^- in pore waters.

b) Analytical chemistry results showed that the addition of fertilizer failed to enhance oil biodegradation, regardless of the application rate. Changes in oil composition occurred, although slowly, but were similar for both treated and untreated plots. It is not known what conditions at the Disk Island site could have precluded enhanced oil biodegradation. Disk Island may represent a type of beach (low energy, low slope profile, less porous beach material) that is not amenable to oil bioremediation because of insufficient oxygen availability and/or high natural organic matter content (peat deposits). However, stable

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carbon isotope analysis in microcosm experiments demonstrated that oil carbon was a substantial proportion of the total carbon supply to the bacterial assemblage.

c) Measurements of oil mineralization, based on total CO_2 production or $^{14}CO_2$ released from radiolabeled hydrocarbons (phenanthracene and hexadecane), did show effects due to fertilizer application rate. It was quite evident that considerable oil biodegradation was occurring in the beach samples. Production of large amounts of radiolabeled CO_2 from phenanthracene and hexadecane strongly suggested that this was due to biodegradation of oil and not other types of organic matter (humic materials). Adding fertilizer generally caused significant increases in mineralization rates relative to the untreated control plots, with the greatest stimulation occurring with the 500 g/m^2 application (approximately 2 to 5 times greater than the untreated controls). The largest application (1,000 g/m^2) actually seemed to inhibit mineralization to some extent. A calculated dose response indicated that a six-fold increase in fertilizer granule application produced a two-fold increase in oil degradation rates. In addition, oil-degrading bacteria increased following fertilizer application. Concentrations were almost 10-fold greater than the untreated controls on the plots receiving the two highest fertilizer concentrations.

d) Stable carbon and nitrogen isotopes were used to trace nitrogen from bioremediation treatments into intertidal beach food chains and to examine trophic structures. This assimilation of fertilizer nitrogen was species-specific and related to the proximity of the organism to the treated plot. Stable nitrogen isotope analysis of heterotrophic organisms revealed that the fertilizer was assimilated into the food chains, and assimilation depended on the feeding strategy of the organism. While nitrogen from fertilizer was assimilated into beach food chains, no adverse effect to the food chain structure was observed.

Elrington Island

This field study focused on optimizing the fertilizer solution application used in Passage Cove the previous summer. The effectiveness of multiple pulse doses of fertilizer solution was evaluated against a single pulse dose, and bioremediation of subsurface oil was also examined.

a) It was concluded that bioremediation of subsurface oil was reasonable, if sufficient quantities of nitrogen and phosphorus nutrients can be supplied. This was accomplished by using fertilizer solution.

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b) A single pulse application of fertilizer (4 hours, once at low tide) enhanced oil biodegradation for as long as 3 to 4 weeks. This application was as effective, if not more so, than a multiple dose application. These results raise the question of whether the effects of fertilizer application during the summer of 1989, which also consisted of large initial pulses followed by a gradual release over time of nutrients at lower concentrations, may have been related more to the extent of nutrient exposure to the microbial communities at one time than the length of exposure.

c) Fertilizer-enhanced oil biodegradation rates on Elrington Island were the highest recorded in the field demonstrations. These rates were approximately 100 mg/kg of beach material/day, almost six-fold higher than the untreated Control beach. This occurred despite the fact that rates on the untreated Control beach were approximately three-fold higher than rates reported at Passage Cove the previous summer. This increase in effectiveness of fertilizer application could be due to extensive colonization of the subsurface oil by oil-degrading microorganisms, and/or increased availability of oil to the bacteria by impregnation with glacial till (greater exposed surface area).

d) Measurement of oil mineralization rates in the field and the laboratory using total CO_2 production, oxygen uptake, and nutrient assimilation generally coincided with changes in oil concentration and composition. Relative to oil chemistry analysis, mineralization measurements could therefore provide simpler procedures for assessing the effect of fertilizer-enhanced biodegradation in future field studies.

e) The Elrington Island study clearly validated the use of laboratory oil degradation information from flask and microcosm studies to predict bioremediation events in the field.

f) The use of sampling baskets containing homogenized beach material proved to be a reliable method to supplant direct sampling of beach material. It considerably reduced sampling variability and provided information that was representative of the beach it modeled.

g) Stable isotope studies were also conducted on Elrington Island and compared to results on Disk Island. On Elrington only one alga, *Fucus distichus*, was observed to assimilate fertilizer nitrogen. Differences between beaches are attributed to a steeper beach slope on Elrington Island, resulting in stronger definition of ecological niches.

Other Studies

a) To substantiate and interpret results obtained in field studies, a series of laboratory research projects were completed during the winter of 1989/1990. These projects included evaluation of application strategies for fertilizers, investigation of the mode of fertilizer action, and bioaugmentation studies. Results from shake flasks and microcosm studies showed that a single pulse of fertilizer nutrients was as effective as multiple pulses for enhancing oil degradation over a three to four week period. This meant that fertilizer application strategies could ultimately be greatly simplified as was demonstrated in the Elrington Island field study. Studies with oleophilic fertilizer verified that the product affected oil degradation primarily through the provision of nitrogen and phosphorus, and not through some alternative supplement. In other studies, several pure cultures with demonstrated ability to degrade Prudhoe Bay crude oil were isolated from oiled Prince William Sound beach material. When high concentrations of these cultures were reinjected into the oiled beach material, greater initial oil biodegradation occurred compared to oiled beach material receiving only nutrients. Thus, bioaugmentation, based on laboratory tests, merited further study.

b) Due to the complicated ecology involved in the biodegradation of oil by natural microbial communities, it was important to develop an initial predictive model to relate responses of the microbial communities to fertilizer application. Laboratory and field data were used to test a simple deterministic model. The results showed that much more information is needed on the production and fate of the microbial biomass to make this modeling approach generally applicable to oil bioremediation.

c) Field evaluation of two commercial bioremediation products yielded inconclusive results. Most of the readily biodegradable compounds in the oil had likely disappeared during the sixteen months that had elapsed between the oil spill and the initiation of the field test. Lacking sufficient substrate, it was not possible to measure significant differences among the treatments.

SECTION 1

INTRODUCTION

BACKGROUND

Major oil spills have galvanized public attention to alternative cleanup technologies. Oil biodegradation in aquatic (marine and freshwater), terrestrial, and groundwater environments has been extensively studied in laboratory systems over the past 20-30 years (Atlas, 1981 and 1984; National Academy of Sciences, 1985; Leahy and Colwell, 1990; and Bartha, 1986), but it is only recently that this information has been considered for large-scale bioremediation efforts in aquatic environments (Nelson et al., 1987; Bartha, 1986; Morgan and Watkinson, 1989; and Lee and Levy, 1989). Definitive success in the restoration of gasoline-contaminated aquifers (Raymond et al., 1976 and 1978; Minugh et al., 1983; Yaninga et al., 1985; and Brown et al., 1985) and oil-contaminated soils (Bartha, 1986; Rittmann and Johnson, 1989; and Dibble and Barth, 1979) has, however, shown the usefulness of bioremediation and has indicated the importance of a basic research database.

Enhancing biodegradation processes to assist in the cleanup of oil spills in marine environments has been suggested several times, with much emphasis on the treatment of oil on open waters (Lee and Levy, 1989; Halmo, 1985). Several approaches have been discussed and debated, including accelerating oil biodegradation rates by increasing the availability of oil to bacteria through the use of dispersants and seeding oil-contaminated areas with hydrocarbon-degrading bacteria. Both approaches have had mixed results, and the complex logistics of open-water monitoring has made assessments of success ambiguous and inconclusive.

The simplest approach for enhancing oil biodegradation is the addition of nitrogen and phosphorus nutrients in a well-oxygenated environment. It is well known that enrichments of oil-degrading microorganisms occur rapidly following oil spills in most environments. But with a large amount of degradable oil carbon present, biodegradation quickly becomes limited by nutrient and oxygen availability (Lee et al., 1988; Atlas, 1981 and 1984; National Academy of Sciences, 1985; Leahy and Colwell, 1990; Bartha, 1986; and Morgan and Watkinson, 1989). Numerous laboratory and field studies have shown that attempts to overcome these limitations generally lead to successful optimization of oil biodegradation rates and extents (Nelson et al., 1987; Atlas, 1984; Leahy and Colwell, 1990; Morgan and Watkinson, 1989; and Lee and Levy, 1989). This approach has never been used before on a large scale to directly assist in cleanup operations following a major oil spill.

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INTRODUCTION

On March 24, 1989, the *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound, Alaska, releasing approximately 11 million gallons of Prudhoe Bay crude oil. The oil spread onto an estimated 1,000 miles of shoreline (350 miles in Prince William Sound). The oil spill provided a unique opportunity to test the feasibility of bioremediation on a large scale (EPA ORD, 1989), since Prudhoe Bay crude oil had been the focal point of several previous biodegradation studies in cold water environments (Atlas and Busdosh, 1976; Fedorak and Westlake, 1981; Horowitz and Atlas, 1977; Atlas et al., 1978; and Cook and Westlake, 1974).

DESCRIPTION OF THE AREA IMPACTED BY THE SPILL

The site of the *Exxon Valdez* oil spill is a harsh and diverse environment with poor accessibility. The shoreline is geologically young, composed largely of metamorphic rock, and ranges from vertical cliffs to boulder and pebble beaches. High-energy beaches are common, with tides ranging from +4 to -1 m. In some areas, glacial and snow melt introduce large amounts of fresh water to nearshore waters of Prince William Sound. The Sound has a considerable population of seals and sea otters, extensive herring and salmon spawning areas, and significant numbers of seabirds and shorebirds. There is a substantial migration of birds that feed at beaches and intertidal areas.

Major contaminated shoreline areas included Knight Island, Eleanor Island, Smith Island, Green Island, and Naked Island (Figure 1.1 A and B). Knight Island, the largest and one of the most heavily polluted of these islands, has restricted tidal flushing action in some bays and coves. The oil settled into the beach gravel, on rock surfaces, and on the faces of vertical cliffs. Contamination occurred primarily in the intertidal zone.

Initial weathering resulted in a loss of approximately 15% to 20% of the oil mass by volatilization. Volatilized components included normal aliphatic hydrocarbons of less than 12 carbon atoms and aromatic hydrocarbons such as benzene, toluene, xylene, and some methyl-substituted naphthalenes. The resulting residue consisted of alkanes, branched alkanes, heterocyclic chemicals, multi-ring aromatic compounds, high-molecular-weight waxes, and asphaltenes. On most beaches in Prince William Sound the weathered oil was black and viscid and not brown and mousse-like.

Beaches were physically cleaned by Exxon using a combination of flooding and application of water under high pressure and high temperature (140°F) (Figure 1.2). The extent of physical washing was dependent upon the degree of contamination. Vacuum extraction and physical skimming were

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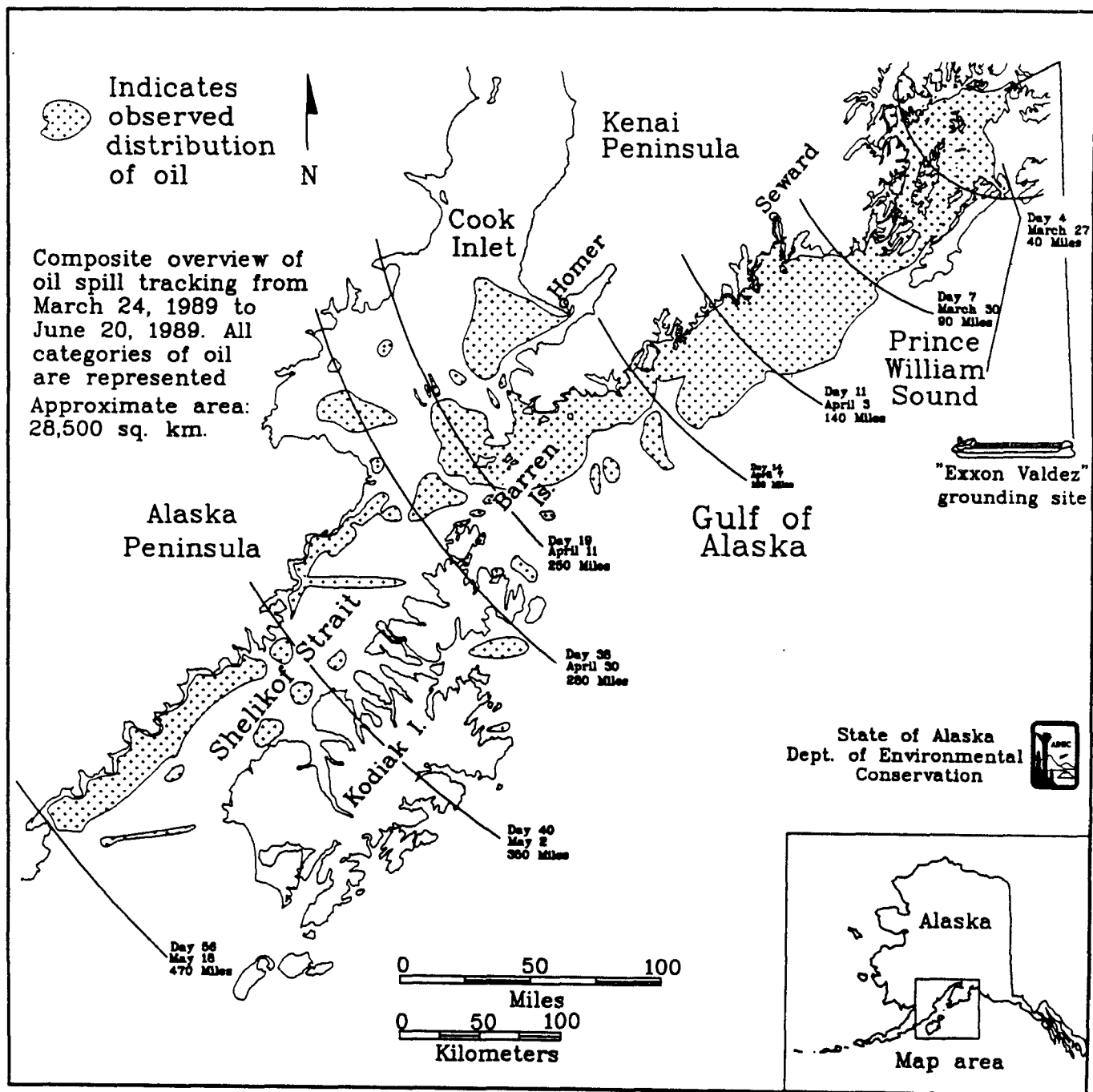
Figure 1.1A.   Diagram of the Oil and Its Impact.

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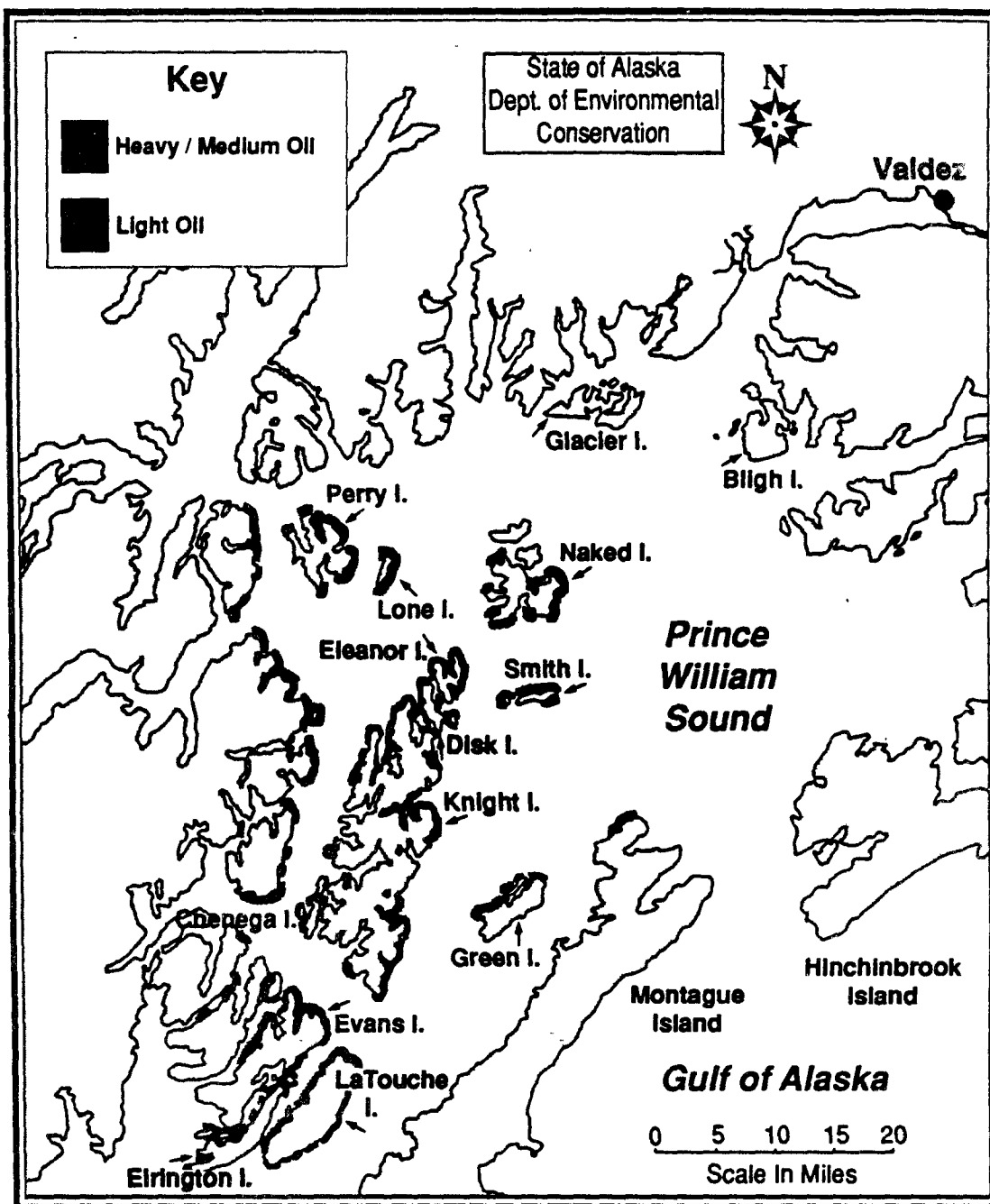
Figure 1.1B.   Oil Impacted Areas in Prince William Sound.

The map shows the following labeled features:

Key
- Heavy / Medium Oil
- Light Oil

State of Alaska Dept. of Environmental Conservation

N

Valdez

Glacier I.

Bligh I.

Perry I.

Naked I.

Lone I.

Eleanor I.

Smith I.

Prince William Sound

Disk I.

Knight I.

Chenega I.

Green I.

Evans I.

LaTouche I.

Montague Island

Hinchinbrook Island

Gulf of Alaska

Elrington I.

0   5   10   15   20
Scale In Miles

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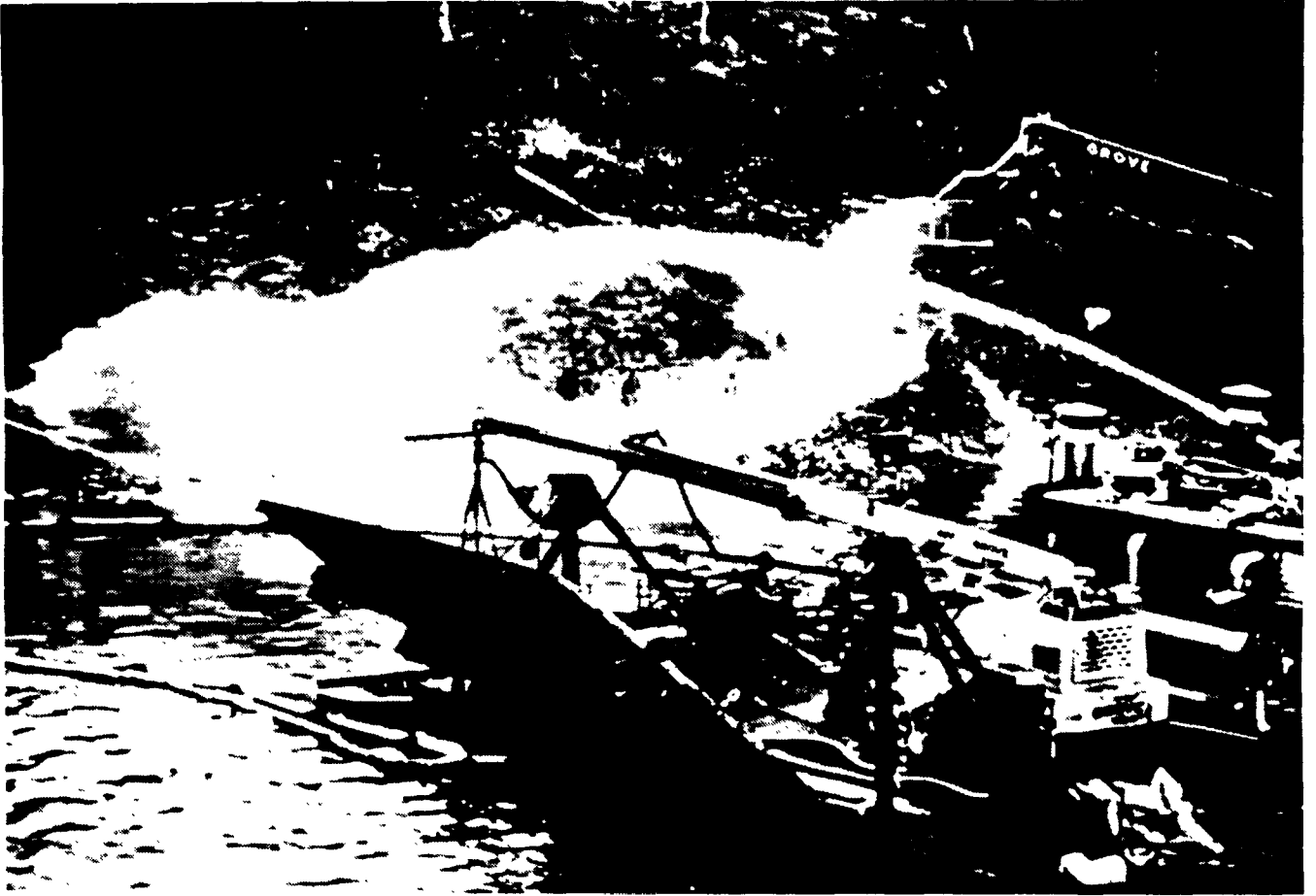
Figure 1.2. Exxon Cleaning a Beach Using Water Under High Pressure and Temperature.

used to remove the released oil from the water surface. The cleaning process partially removed oil from the surface of rocks and beaches, particularly pools of oil, but did not effectively remove the oil trapped in and below the matrix of gravel and cobble. The washing process also spread a thin layer of oil over a greater surface area of rock and gravel.

The composition of oil found on the beaches following this washing (weathered oil) is commonly measured by extracting the oil from beach material and analyzing it with gas chromatography. A typical gas chromatogram of fresh and weathered Prudhoe Bay crude oil is shown in Figure 1.3. The weathered crude oil was taken from a Prince William Sound beach (Northwest Bay) in late spring 1989. The major peaks represent detector responses for the normal alkanes; the annotated numbers are the carbon lengths of the appropriate alkane. Normal aliphatic hydrocarbons of 12 carbons or less are absent in the weathered oil, while large quantities of biodegradable hydrocarbons (nC13-nC28) remain. Gas chromatograms for oil samples fractionated into the aliphatic and aromatic components are shown in Figures 1.4 and 1.5. These fractionated samples of oil showed the presence of small quantities of aromatic hydrocarbons in the weathered oil, but hydrocarbons up to the methyl naphthalene were absent.

Pristane and phytane, branched alkanes, are generally recognized to slowly biodegrade relative to straight chain alkanes (Atlas, 1981) and have thus been used as conserved internal standards to measure biodegradation. Changes in the ratios of hydrocarbon concentration for the linear alkanes relative to the branched alkanes can be used to indicate biodegradation since changes brought about by other physical and chemical processes will not differentially affect the fate of these two hydrocarbon types (Pritchard and Costa, 1991). Table 1.1 gives the calculated ratios of nC17 linear alkane to pristane and nC18 linear alkane to phytane for samples taken from Prince William Sound from April 4 to May 2, 1989. Relative to fresh Prudhoe Bay crude oil, biodegradation of the oil at most beaches had not occurred. The sample from Disk Island (gravel) was the only one with a significant difference in these ratios relative to fresh Prudhoe Bay crude oil. This suggested natural biodegradation was probably occurring at this beach.

BIOREMEDIATION APPROACH

After learning of the magnitude of the spill, the EPA Assistant Administrator for the Office of Research and Development (ORD) convened a meeting of nationally and internationally recognized scientists in the field of oil biodegradation in April, 1989, to evaluate the feasibility of using
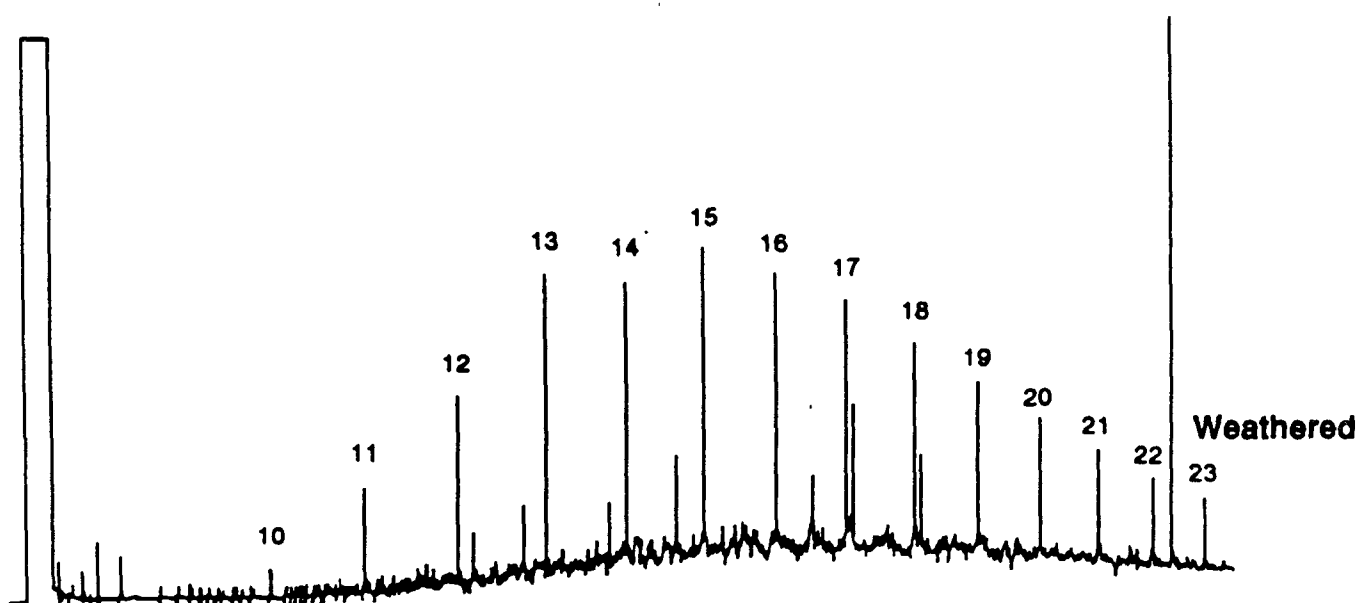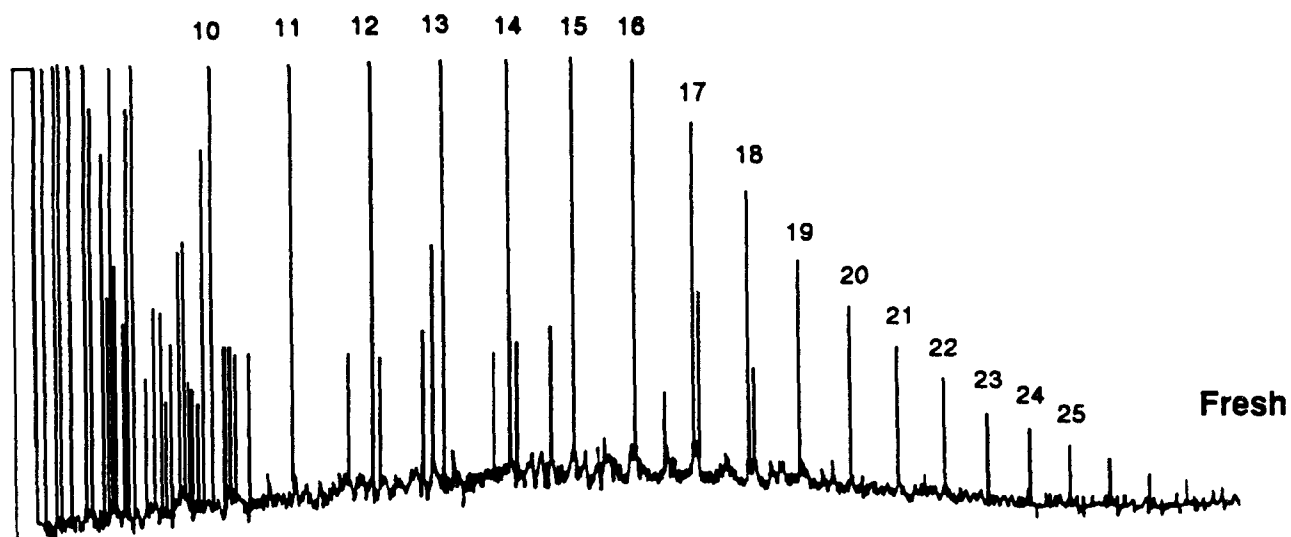
Figure 1.3. Unfractionated Prudhoe Bay Crude Oil (Number Indicates Carbon Atoms of Alkane).
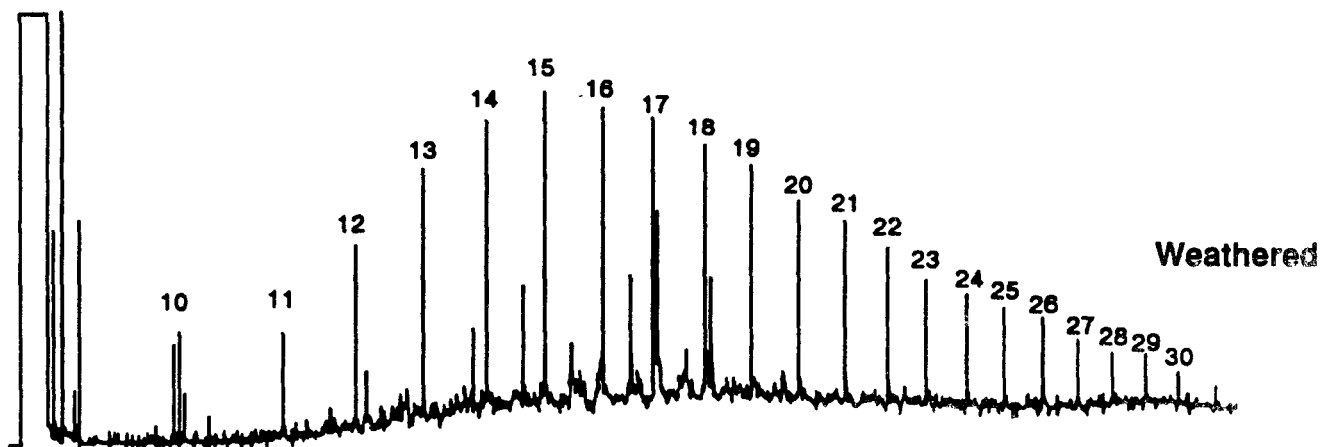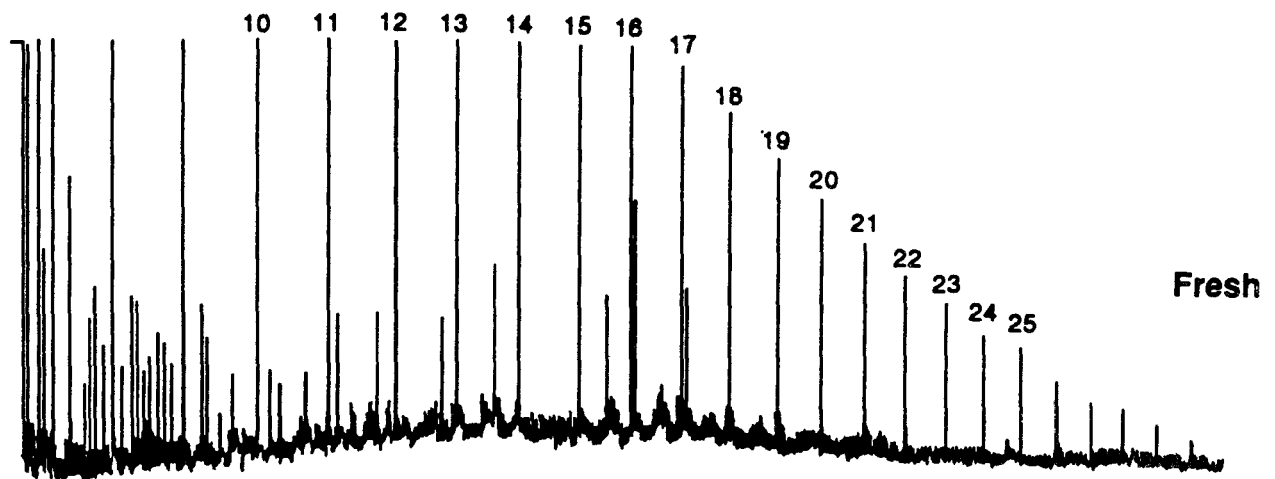
Figure 1.4 Prudhoe Bay Crude Oil, Aliphatic Fraction (Number Indicates Carbon Atoms of Alkane).
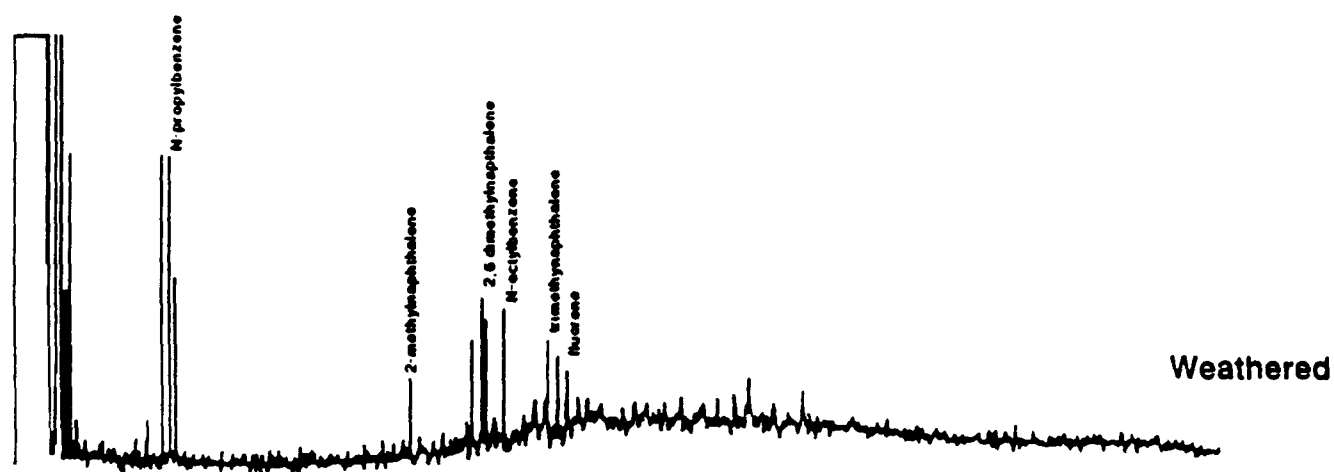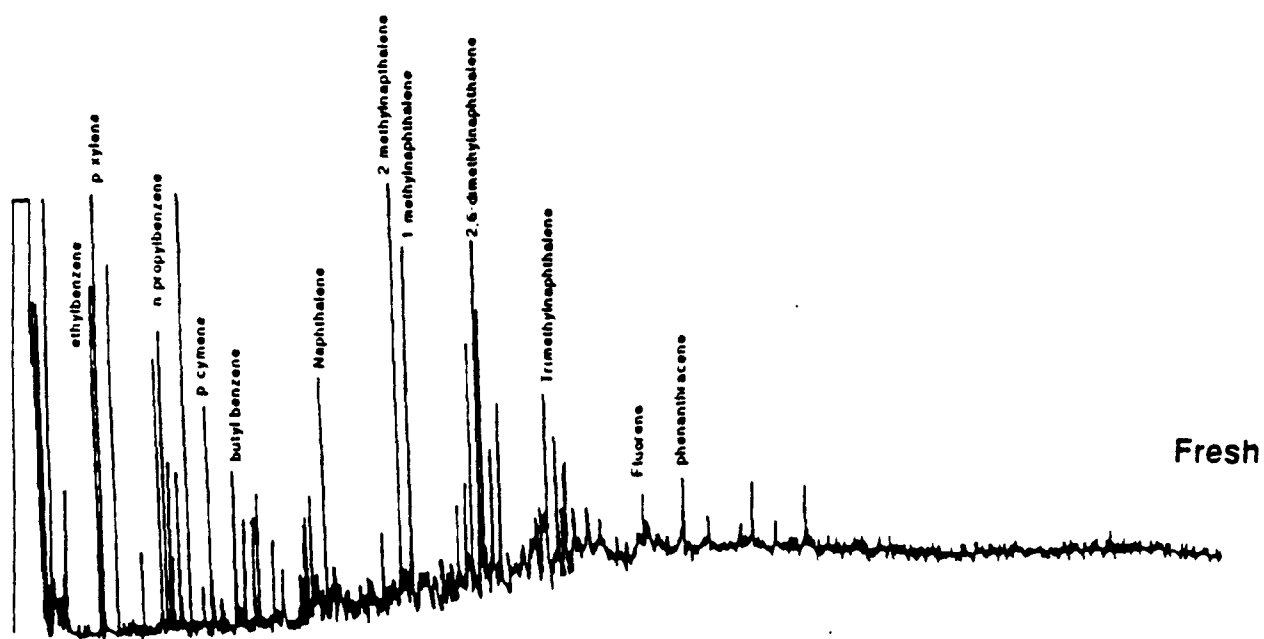
Figure 1.5.   Prudhoe Bay Crude Oil, Aromatic Fraction.

TABLE 1.1. CALCULATED RATIOS OF NC17/PRISTANE AND NC18/PHYTANE

Sample	nC17/ Pristane	nC18/ Phytane
Fresh Prudhoe Bay Crude Oil	1.7	2.0
Eleanor Island		
Northwest Bay		
Surface	1.5	1.9
Surface Control[a]	<0.47	--
6" Depth	1.4	1.7
6" Depth Control[a]	<0.45	--
Seal Island	1.6	2.1
Smith Island	1.5	1.9
Disk Island		
Gravel	0.8	1.0
Fresh Oiled Rock	1.4	1.7
Weathered Oiled Rock	1.8	2.0

[a] Sample taken from an uncontaminated beach area.

bioremediation to assist cleanup operations. The scientific group recommended that ORD plan and conduct a field demonstration project to evaluate the use of fertilizers for accelerating natural biodegradation of the spilled oil in Prince William Sound. This recommendation was based on the following conclusions:

- The presence of readily degradable hydrocarbons from the spilled oil would enrich naturally occurring oil-degrading bacteria.

● Oil biodegradation in Prince William Sound waters was probably limited by the availability of nitrogen and phosphorus; therefore, fertilizing the beaches with these nutrients would enhance natural degradation of the oil.

● Past studies have convincingly shown that enhancement of oil biodegradation readily occurs through nutrient addition. Further verification of these studies by laboratory experiments was unnecessary.

● Successful bioremediation would require consideration of the logistics and mechanics of long-term nutrient application and the physical agitation of oil.

● An oleophilic fertilizer, such as produced by Elf Aquitaine Chemical Company, may be the only way to assure extended contact of the nutrients with the oil-contaminated beach material.

● Bioremediation should be used as a finishing step for any cleanup program. Once the bulk oil was removed (regardless of the method), bioremediation would further reduce the amount of residual oil.

● Treatment of the beaches with fertilizer would not necessarily remove all residues (i.e., little visual improvement) but it would considerably reduce, if not eliminate, ecological availability of the oil.

● Inoculation of oil-contaminated beaches with hydrocarbon-degrading microorganisms enriched from Prince William Sound waters was considered inappropriate as an initial approach but should be considered, in an experimental context, for future spills.

EPA, having a large research program in bioremediation (EPA ORD, 1990-600/9-90/041), including the necessary technical personnel and in-house contractors, consequently responded rapidly to the workshop recommendations.

A bioremediation research project was thus initiated. The goal was to perform an initial field demonstration of bioremediation as a cleanup tool, and, if successful, make recommendations for wider-scale use to Exxon. In addition, EPA would provide a follow-up field study to large-scale application as definitive indication of bioremediation success. A research plan was developed containing the following objectives:

● To examine the rate and extent of natural biodegradation on oil-contaminated beaches.

● To determine if oil biodegradation rates on oil-contaminated beaches could be enhanced by the addition of nutrients in the field to merit the use of bioremediation as a cleanup tool.

● To develop methods for long-term application of nutrients to oil-contaminated beaches.

- To establish methods for monitoring potential ecological effects resulting from nutrient addition.

- To develop information on the movement of nutrients in beach substrata (beach mechanics).

- To examine the possibility of enhancing oil biodegradation through microbial inoculation.

The plan was reviewed by a special committee of EPA's Scientific Advisory Board, and they recommended implementation with minor modifications.

Following development of the research plan, a cooperative effort was proposed to Exxon under the Federal Technology Transfer Act of 1986. On June 2, 1989, the two parties reached an agreement, and the project was formally initiated. Exxon agreed to provide all logistical support (transportation from Valdez to test sites, field laboratory facilities, and subsistence) and $1.6 million for direct support of the field demonstration project. EPA provided $1.6 million for management personnel, scientific expertise, quality assurance, and operations technical support.

A team of experts from the different research laboratories within the ORD (see Appendix A for listing) was assembled to implement the field demonstration project. A brief overview of the major events is given below. A more detailed chronology of events is given in Appendix B.

FIELD OPERATIONS - SUMMER 1989

Field operations began in early May 1989, using the mobilization capability of ORD laboratories at Las Vegas, NV; Gulf Breeze, FL; Cincinnati, OH; Athens, GA; Research Triangle Park, NC; and Ada, OK (see Appendix A for support personnel). It was imperative to initiate the field demonstration as quickly as possible to provide enough time during the summer for large-scale application if the results were favorable.

Test Beach Selection

Test beaches at Snug Harbor and Passage Cove on Knight Island were selected for testing during the summer of 1989. These beaches were mainly comprised of large cobblestone overlying a mixed sand and gravel base. The Snug Harbor beaches had a moderate degree of oil contamination confined to a broad band within the intertidal zone. At the initiation of the project, oiled beaches that had been physically washed by the Exxon process were not available. Thus, the Snug Harbor study site

was chosen to approximate beach conditions following physical washing. Passage Cove, a more heavily oiled beach, was selected later in the summer after it was physically washed by Exxon. Physical washing resulted in the removal of the bulk oil and spread the remaining oil over the beach surfaces. Both beaches had a thin layer of oil covering the surface of the cobblestone, as well as oil mixed into the sand and gravel under the cobblestone to varying depths.

Fertilizer Selection

The spill situation necessitated rapid evaluation and selection of fertilizers, and was based on considerations of application strategies, logistical problems for large-scale application, commercial availability (particularly if large-scale application became reasonable), and the ability to deliver nitrogen and phosphorus nutrients to the microbial communities on the surface and subsurface beach material for sustained periods of time. Three fertilizer application strategies were adopted for testing: commercially available slow-release formulations, an oleophilic fertilizer, and fertilizer solution (Rogers et al., 1990).

Snug Harbor Demonstration

Initial field fertilizer application was conducted at Snug Harbor in June using oleophilic and slow-release fertilizers. Effects of the fertilizer applications on the oil-degrading microbial communities in the beach material were examined. Oil biodegradation was tracked through time using analytical chemistry (to determine changes in oil residue weight and composition) and microbiological techniques. In addition, visual observations were also recorded.

A monitoring program was established to investigate potential adverse ecological effects and verify the safety of bioremediation as a cleanup tool. The potential for eutrophication was investigated through measurements of ammonia, phosphate, chlorophyll, bacterial abundance and productivity, and phytoplankton primary productivity. To address the question of physical removal of the oil (as opposed to biodegradation), caged mussels were placed offshore and sacrificed for oil concentration measurements.

In addition to the field testing, laboratory and microcosm experiments were designed to supplement the field activities. Potential mutagenicity activity associated with the biodegradation of oil was investigated, and incorporation of applied fertilizers into the food web was assessed using stable isotopes.

INTRODUCTION

By the middle of July 1989, results from the field demonstration project convinced Exxon to use large-scale bioremediation as part of their cleanup effort. Exxon began fertilizer application in early August to approximately 70 miles of physically washed beach in Prince William Sound. Increasing the biodegradation rate of oil at this point was very important in order to achieve maximal degradation before winter conditions curtailed cleanup operations.

Passage Cove Demonstration

The Passage Cove study was initiated as the definitive technical support site for the large-scale application of fertilizers. Nutrient solution and oleophilic plus slow-release fertilizer were applied to Passage Cove in late July 1989. Oil degradation was again assessed using analytical chemistry (changes in oil residue weight and composition) and microbiological techniques (most probable number counts and respiration). Visual observations were again recorded.

The same ecological monitoring program for direct toxicity and eutrophication was implemented, and possible adverse ecological effects resulting from direct toxicity of the fertilizer were also addressed. Caged mussels were again placed offshore of the field site to evaluate physical removal of the oil.

Laboratory tests conducted to supplement the field testing at Passage Cove included: 1) use of microcosms to test the effectiveness of subsurface oil bioremediation under more controlled conditions than the field; 2) assessment of the incorporation of fertilizers into the food web using stable isotopes; and 3) measurements of microbial activity at Passage Cove.

Supporting Laboratory Studies

In addition to the laboratory studies conducted to specifically supplement the activities at the two field sites, experiments were also conducted to investigate more general parameters. These included: 1) laboratory biodegradation screening evaluations to determine if degradation in Prince William Sound was limited by nutrient availability; 2) respirometric analyses to obtain additional information on the effect of oleophilic fertilizer for enhancing the degradation of different concentrations of artificially weathered oil; 3) experiments to determine the mechanism by which oleophilic fertilizer enhanced oil degradation; 4) tests to evaluate the rock-washing characteristic of

oleophilic fertilizer under conditions that precluded biological activity; and 5) acute toxicity tests of oleophilic fertilizer to fish, invertebrates, and algae.

Follow-Up Research - Winter 1989/1990

The bioremediation research conducted during the summer of 1989 showed enhancement of biodegradation through specific introduction of fertilizers. Several major problems, however, needed to be addressed to assist in interpreting these results and assessing future fertilizer applications. Laboratory studies were therefore conducted during the winter of 1989/1990 to complement the summer of 1989 research and provide insight for the summer of 1990 field studies. Three study areas were examined during the winter of 1989/1990:

- Mechanism of action of oleophilic fertilizers and optimization of fertilizer application for maximal rate of oil degradation;

- Toxicity of fertilizers to marine biota; and

- Eutrophication modeling in select bays and areas of Prince William Sound

Studies on the mechanism of action of oleophilic fertilizers were conducted using microcosms. Changes in oil composition were used as the indicator of oil degradation. Experiments for optimizing fertilizer application included fertilizer-specific activity studies and alternative treatment application scenarios.

Additional sampling was conducted in Passage Cove in November 1989, sediment chemistry data were obtained and oil degradation rates were analyzed. Tests were conducted on compounds such as oleic acid or laureth phosphate, and initial studies with a soybean oil product for enhanced degradation rates.

To determine the toxicity of fertilizers to marine biota, the relative toxicity of urea/ammonia and lauryl phosphate was examined in standard laboratory tests. Acute and chronic toxicity tests were conducted on several INIPOL exposure regimes, and a literature review was conducted on ammonia toxicity to marine invertebrates.

To investigate the possibility of eutrophication, a eutrophication model, EUTRO4, was instituted for Passage Cove and Snug Harbor. Residence times for nutrients in both bays were calculated, and

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eutrophication potential was determined. A number of nutrient loading scenarios were tested to determine the potential for eutrophication under worst case conditions.

FIELD OPERATIONS - SUMMER 1990

Monitoring Program

As a result of summer 1989 and winter 1989/1990 studies, the 1990 cleanup plan for the remaining oil-contaminated shorelines in Prince William Sound that was agreed upon by the U.S. Coast Guard, Exxon, EPA, the Alaskan Department of Environmental Conservation (ADEC), and other resource agencies and landowners involved bioremediation as an integral part, both as a primary and secondary cleanup method. However, further substantiation of bioremediation effectiveness was required.

To provide this information, a joint bioremediation monitoring program was conceived and implemented by scientists from Exxon, EPA, ADEC, and the University of Alaska using Exxon logistical and resource support. Three beaches that were part of the cleanup plan were selected for monitoring. Changes in oil chemistry, dissolved oxygen, and nutrient concentrations were monitored in surface and subsurface samples, as well as increases in the number of oil degraders, hydrocarbon mineralization activity, and toxicity to bioassay test species. The results from this study are reported under separate cover.

To complement this monitoring program, additional experimental research in the field was also planned. The intent of the research was to strengthen the success of bioremediation and optimize its effectiveness under different field conditions. The beaches selected for experimentation were Disk Island and Elrington Island. Additional laboratory studies were conducted to complement and verify the field studies.

Disk Island Demonstration

The Disk Island study was designed to determine the specific activity of fertilizer-enhanced biodegradation, or the extent of rate enhancement per quantity of nutrients released. To evaluate the effect of applying different concentrations of fertilizer on biodegradation, samples were taken for

oil chemistry (changes in oil residue weight and composition), increases in oil-degrading microbial activity and biomass, and resulting nutrient concentrations.

A scaling experiment was also conducted at an uncontaminated cobble beach in association with the Disk Island study, to assure that the application of fertilizer granules would release nutrients into a defined plot size. Subsurface wells were used to measure nutrient release following fertilizer application.

Elrington Island Demonstration

The Elrington Island study was initiated to investigate the effects of different applications of nutrient solution on stimulating oil biodegradation. To assess potential increases in biodegradation, analytical chemistry analyses (changes in oil residue weight and composition), increases in oil-degrading microbial activity and oil-degrader biomass, dissolved oxygen uptake, and uptake of inorganic nutrients were evaluated. The ability of bioremediation to work effectively on oil in the beach subsurface (0.3 to 1.0 m depths) was emphasized.

SECTION 2
FERTILIZER SELECTION AND CHARACTERISTICS

BACKGROUND

An important aspect of this project was the selection of fertilizers for the field tests. The goal was to find fertilizer formulations which would either slowly release nitrogen and phosphorus nutrients or keep the nutrients in contact with surface microbial communities for extended time periods. In addition, consideration was given to formulations amenable to practical and inexpensive application to contaminated shorelines, in light of the possibility of future large-scale applications. Three general types of fertilizer were selected for testing:

1) Solid, slow-release fertilizer, in which nutrients would be slowly released from a point source and distributed over the beach surface by tidal action. The product had to deliver sufficient quantities of nutrients for several weeks.

2) Liquid oleophilic fertilizer, in which nutrients would "dissolve" into the oil covering the rock and gravel surfaces. This sequesters nutrients in the oil phase, facilitating bacterial growth on the surface over sustained periods. Nutrient distribution over the beach material would be accomplished in the original fertilizer application.

3) Fertilizer solutions, in which inorganic nitrogen and phosphorus would be dissolved in seawater and applied through spray irrigation (a fixed sprinkler system). This type of application would introduce nutrients into the oiled beach material, particularly oil below the surface, in a defined, controlled, and reproducible way.

Several commercially available fertilizer formulations that satisfied these requirements were selected and their nutrient-release characteristics determined. A small study was also conducted to see how specific solid fertilizer formulations physically behaved under field conditions.

DESCRIPTION OF FERTILIZER FORMULATIONS

The fertilizer formulations selected for initial testing prior to use in the field are described below.

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WOODACE Briquettes

This fertilizer formulation contains isobutyraldehydediurea (IBDU),[1] a chemical that spontaneously hydrolyzes into isobutylaldehyde and urea. This process is responsible for the characteristic slow release of nitrogen. Hydrolysis is temperature dependent, and while slower at lower temperatures is still significant. The source of phosphorus is Linstar, a citric acid soluble phosphatic fertilizer developed by Mitcubichi Chemical Corp. Each briquette weighs approximately 17 grams, on average, and has a specific gravity of 1.5 to 1.8. This fertilizer has an N:P:K ratio of 14:3:3.

PAR EX Granules

This fertilizer is granular, with an N:P:K ratio of 24:4:12, formulated to give an immediate and sustained release of nutrients. The product used was PAR EX, produced by Estech, Inc. All of the nitrogen is derived from ammonium phosphate, urea, and IBDU. A minimum of 45% of the nitrogen is derived from the IBDU. The available phosphorus is derived from potassium magnesium phosphate and iron is also present as ferrous sulfate. The granules have a specific gravity of approximately 1.3.

OSMOCOTE Briquettes

Manufactured by SIERRA CHEMICAL, this fertilizer contains urea formaldehyde as the nitrogen source in a slow-release formulation created by thermoplastic resin (natural plant product binder) encapsulation. The urea formaldehyde released from the briquettes must be biologically hydrolyzed to produce ammonia. Phosphorus is present as calcium phosphate and iron is also present as ferrous sulfate. Each briquette weighs 21 grams, on average, and the fertilizer N:P:K ratio is 20:10:5.

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[1]Isobutyraldehyde Diurea (IBDU) is a condensation product of urea. The reaction can be carried out both in aqueous solution and between solid urea and liquid aldehyde as follows:

$$\begin{array}{ccc} CH_3 & CH_3 & NHCONH_2 \\ > CHCHO + 2\ NH_2CONH_2 = & > CHCH < & + H_2O \\ CH_3 & CH_3 & NHCONH_2 \end{array}$$
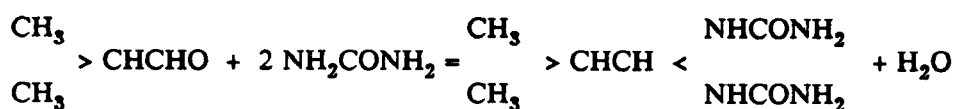
MAGAMP

This fertilizer formulation contains magnesium ammonium phosphate (MAGAMP), which is sparingly soluble in water. It is made by Martin Marietta Magnesia Specialties using specialized manufacturing procedures. The fertilizer can be cast into different shapes (granules, briquettes, bricks) of varying densities, and will slowly release ammonia and phosphate when submersed. This fertilizer has an N:P:K ratio of 7:40:0 and is available in briquettes that weigh 209 g each, on average, and bricks of 8 or 40 lbs.

SIERRA CHEMICAL Granules

This fertilizer formulation (also referred to as CUSTOMBLEN) consists of inorganic nutrient sources (ammonium nitrate, calcium phosphate, and ammonium phosphate) contained in a vegetable oil coating (polymerized by reaction with a diene). The coating gives the fertilizer its slow-release characteristic. The N:P:K ratio is 28:8:0 and the granules have a specific gravity of approximately 1.8.

INIPOL Oleophilic Fertilizer

The only oleophilic fertilizer available in sufficient amounts to use in a scaled-up operation was the Elf Aquitaine (France) product, INIPOL EAP 22. This is a mixture of nutrients encapsulated by oleic acid (the external phase). It is theorized that oleic acid and surfactants in the fertilizer formulation cause the nutrients to become sequestered in the oil phase, preventing rapid release of the nutrients into the aqueous phase and subsequent washout. INIPOL is a yellow liquid with a specific gravity of 0.996, a viscosity of 250 cSt, a pour point of 11°C, and a flash point of >100°C. The N:P:K ratio is 7.3:2.8:0.

The main ingredients in INIPOL are oleic acid and urea, plus chemicals to maintain them in a microemulsion. The chemical composition of INIPOL is given in Table 2.1. The product is designed to initially stimulate oleic acid-degrading bacteria. The quantity of nitrogen and phosphorus present is sufficient to allow the natural oleic acid degraders found in the receiving environment to consume all of the oleic acid carbon present in the INIPOL. Once the added oleic acid is consumed, and the numbers of oleic acid-degraders have increased substantially, oil biodegradation is thought to commence. It is not exactly clear why this pattern occurs, but many oleic acid-degrading bacteria are known to degrade petroleum hydrocarbons. Elf Aquitaine representatives have suggested that the

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oleic acid-degrading microorganisms may die once they reach a certain density, creating a natural recycling of nutrients through mineralization of this dead biomass.

TABLE 2.1. INIPOL EAP 22 CHEMICAL COMPOSITION

Chemical	Purpose
Oleic acid	Oleophilic Phase (Continuous)
	Primary Carbon Nutrient
Tri[laureth-4]phosphate	Phosphate Nutrient Surfactant
2-Butoxy-1 Ethanol	Co-Surfactant Emulsion Stabilizer
Urea	Nitrogen Nutrient
Water	Hydrophilic Phase

All of these fertilizers were tested in the laboratory, field, or both in the summer of 1989 prior to field application, in order to determine their nutrient release characteristics and subsequent suitability for field testing. The methods and results from the tests are presented below.

METHODS

To simulate the effect of tidal activity on the nutrient release characteristics of the selected fertilizers, static and intermittent submersion laboratory tests were developed. These tests were conducted to determine which fertilizers would satisfy the minimum requirements for field testing. The static test represented high tide, submerged conditions when turbulence was at a minimum. The intermittent submersion test represented the turbulent condition, simulating water moving from low to high tide and back. For the INIPOL fertilizer, varying amounts of fertilizer were applied to determine the best application rate and nutrient retention characteristics. Excess INIPOL fertilizer, when not adsorbed to the oiled surfaces, loses its solution properties on contact with water and releases urea very rapidly.

FERTILIZER SELECTION

Static Tests

Tests for granular fertilizers were conducted by sealing a specific weight of the granules in porous, 100% cotton cloth bags, or placing a specific weight of the granules at the bottom of the beaker, and submerging in a beaker of artificial seawater (Instant Ocean, supplied by Aquarium Systems, Inc.). The briquettes were tested by placing them in the beaker and submerging with the seawater. The beakers were maintained at 15°C without mixing. According to an established schedule, water was decanted out of the beakers and replaced with new seawater. Consequently, the effect of the water exchange on the quantity of nutrients released could be assessed. The amount of total Kjeldahl nitrogen (TKN) (EPA method 365.4), ammonia (EPA method 350.1), nitrate (EPA method 353.1), and total phosphorus (EPA method 365.4), were monitored, depending on the fertilizer tested.

To determine the effect of microbial activities on the release of ammonia from the TKN leached out of the IBDU briquettes, flask studies were conducted in which briquettes were covered with three types of water: deionized, sterile seawater (filter sterilized - 0.22μ), and non-sterile seawater. The experiments were run at two different temperatures (9°C and 21°C) and the amount of ammonia released over time determined. To verify that using Prince William Sound beach material would produce similar results, a test was also conducted with the beach material.

The oleophilic fertilizer was tested by applying the fertilizer with an air paint sprayer to the surface of oil-contaminated rocks obtained from Prince William Sound. The rocks were then covered with seawater within 5 minutes and accumulation of TKN, NH_4, NO_3, and NO_2 in the water over time was monitored.

Intermittent Submersion Tests

A rocker table equipped with a 8 cm long, 10 cm wide pipette tray was used for the intermittent submersion tests. A bag containing fertilizer granules or the fertilizer briquettes alone was buried in clean Alaskan beach material at one end of the tray. The air paint sprayer was again used to apply the oleophilic fertilizer directly on the beach material. The rocker table was maintained in a cold room at 15°C, generally operated for 120 minutes, and was sampled for nutrients on a set schedule. The table was then stopped, the water drained, and the beach material allowed to remain undisturbed for 4 hours. The beach material was covered again with water, the table was operated for one hour,

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and sampling was repeated. The process was repeated several times. Other operational variations were tested, depending on the intent of the specific experiment.

Field Tests

To test nutrient release characteristics of magnesium ammonium phosphate (MAGAMP) under field conditions, 8 lb and 40 lb MAGAMP bricks were placed on a sand and gravel beach at Snug Harbor. Sampling stations were placed downgradient of the fertilizer briquettes. The weight of the bricks was expected to minimize their movement by tidal and wave action. Nutrient samples were collected 12, 24, and 96 hours after placing the bricks on the beach.

RESULTS

The results of the field and laboratory tests for the three general types of fertilizers chosen for testing are detailed below. Figure 2.1 diagrams the overall testing scheme for fertilizer selection.

WOODACE Briquettes

The cumulative nutrient release pattern for ammonia, total phosphorus, and TKN from static tests with the IBDU briquettes is shown in Figure 2.2. Release rates per day for each nutrient are shown in Figure 2.3. The release of ammonia from urea will only occur in the presence of a biological agent. Bacteria were not purposely introduced, and consequently ammonia release should be minimal. The release rate was relatively constant after the initial surge, probably caused by the powder on the briquettes. Release of TKN accounted for 17% of the total available nitrogen after 17 days, 31% after 60 days, and 45% after 140 days. Release rates for total phosphorus, although somewhat variable, were constant over time. Total phosphorus continued to be released over the test period, with values averaging approximately 3 mg/L and a cumulative release of 36%. The variability was probably associated with analytical error. The schedule of water exchanges appeared to have a negligible effect on release rates for this fertilizer (data not shown).

It is apparent that small amounts of ammonia and phosphorus are released with each 24-hour soaking of the briquettes. The average amount of ammonia released per day is approximately 100-fold higher than background levels in Snug Harbor waters (see Section 6: Nutrients). However, considering the rapid dilution of ammonia that will occur in the field following release from

Fertilizers Tested

- WOODACE Briquettes
- PAR EX Granules
- OSMOCOTE Briquettes
- MAGAMP
- SIERRA CHEMICAL Granules (CUSTOMBLEN)
- Oleophilic (INIPOL EAP 22)

Static Tests

- WOODACE Briquettes
- PAR EX Granules
- OSMOCOTE Briquettes
- MAGAMP
- SIERRA CHEMICAL Granules (CUSTOMBLEN)
- Oleophilic (INIPOL EAP 22)

Intermittent Submersion Tests

- WOODACE Briquettes
- MAGAMP
- Oleophilic (INIPOL EAP 22)

Field Tests

- WOODACE Briquettes
- MAGAMP

Fertilizers Recommended for Field Testing

- Initial 1989 Field Demonstration (Snug Harbor): WOODACE Briquettes; INIPOL EAP 22
- Second 1989 Field Test (Passage Cove): SIERRA CHEMICAL Granules (CUSTOMBLEN) and INIPOL EAP 22 together
- Initial 1990 Field Test (Disk Island): SIERRA CHEMICAL Granules (CUSTOMBLEN)
- Second 1990 Field Test (Elrington Island): Inorganic Nitrogen and Phosphorus (granular)[a] Mixed with Seawater

  [a] Product Not Tested in 1989 Fertilizer Test Studies

Figure 2.1. Diagram of Fertilizer Testing for Nutrient Release Characteristics.
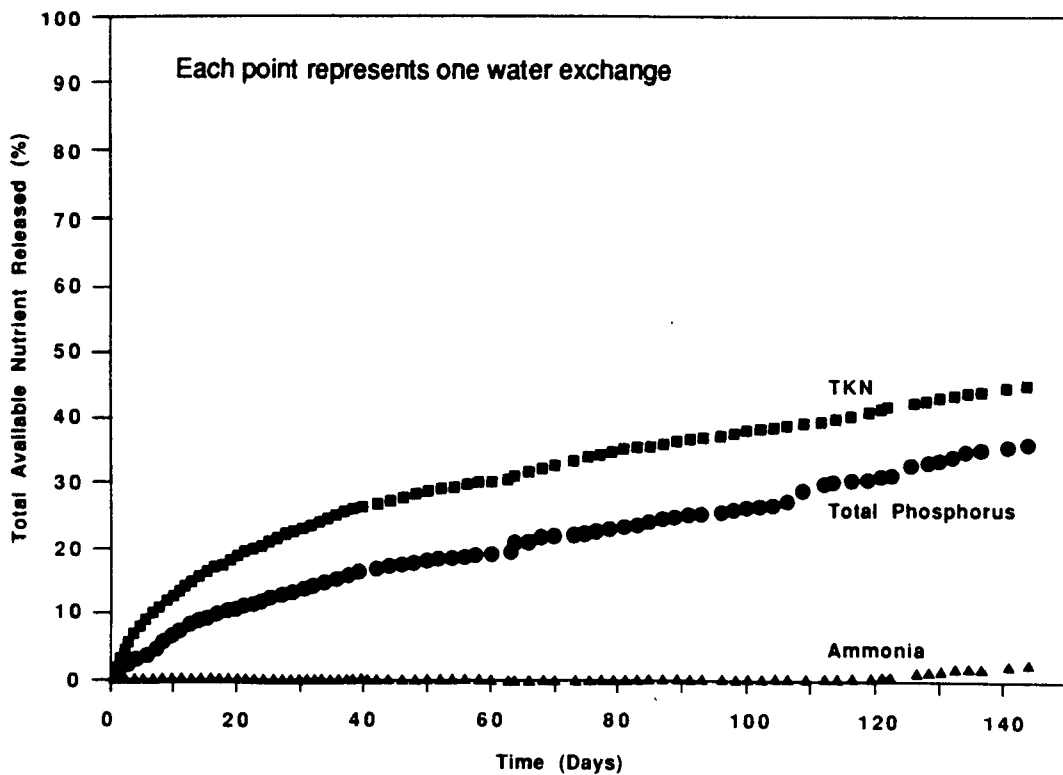
Figure 2.2. Cumulative Release of Ammonia, Total Kjeldahl Nitrogen (TKN), and Total Phosphorus from WOODACE Briquettes in Static Flask Experiments.
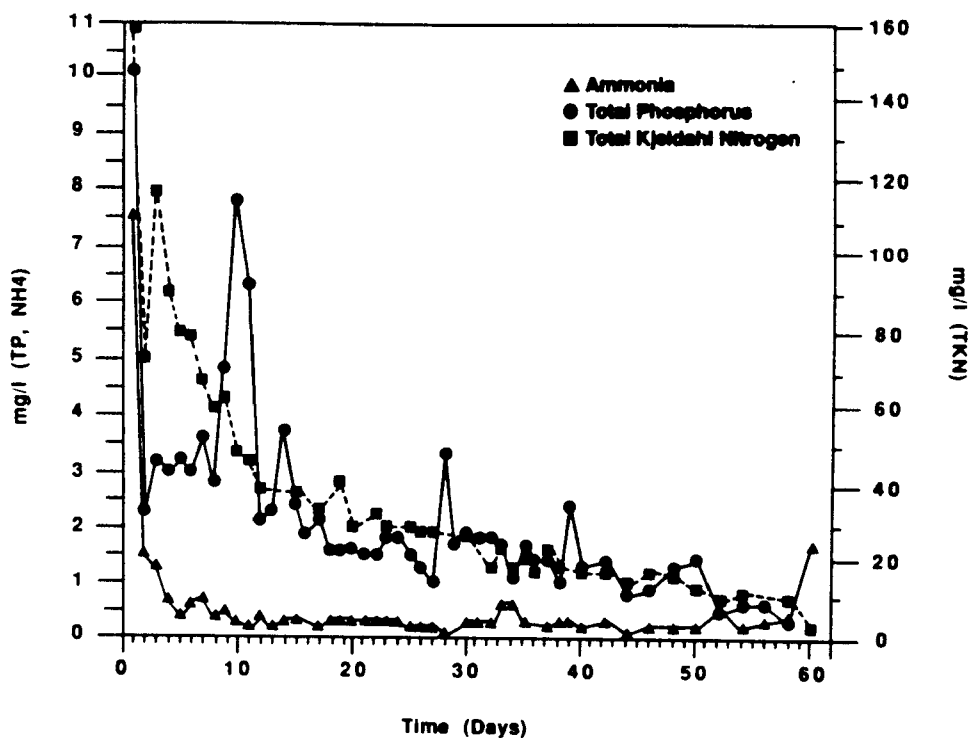
Figure 2.3. Daily Nutrient Release Rate of Ammonia (NH_4), Total Phosphorus (TP), and Total Kjeldahl Nitrogen (TKN) from WOODACE Briquettes.

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briquettes, it would be unlikely to measure any increased concentrations of ammonia (discarding contributions from the TKN) in the field as a result of fertilizer application. The total amount of ammonia released is only a small fraction of the total nitrogen available in the formulation.

To determine if the TKN was a source of ammonia to bacteria under natural conditions, briquettes were soaked for 3 succesive 1-hour periods (water was changed for each period) using 2 different temperatures and 3 different sources of water. The results are shown in Figure 2.4. Significant amounts of ammonium were released under all conditions. The lowest amount of ammonia was released with filtered seawater (containing no microorganisms). Since this was less than that released in the presence of deionized water, it suggests that there was an ionic strength effect on the ammonia release. Temperature had little effect on release rates in these media. However, with unfiltered seawater, considerably more ammonia was released, particularly at the higher temperatures. This indicated a possible biological effect on ammonia release, presumably through the microbial breakdown of the TKN fraction.

Nutrient release from the IBDU briquettes was also tested using the intermittent submersion test. In general, concentrations of the nutrients and TKN released were minimal.

Under all conditions, the physical integrity of the IBDU briquettes was excellent, with very little change in shape and consistency after being submersed in water for four months. A simple freeze/thaw experiment was also conducted on the WOODACE briquettes. The experiment consisted of alternately freezing and thawing submerged and non-submerged briquettes, then weighing and visually observing changes. The results indicated good durability. The briquettes appeared to be a good choice for field application.

Studies were also performed on the movement of briquettes broadcast on the beach. Results showed that unconfined briquettes will not retain their original position and were redistributed after several tidal cycles. Greater redistribution occurred on sand and gravel beaches as compared to cobble beaches. Due to the redistribution of briquettes, this form of fertilizer application is best applied in containers which hold the briquettes in place. Unconfined briquettes may be of some limited use on sheltered cobble beaches, where wave action may have less influence on the beach.
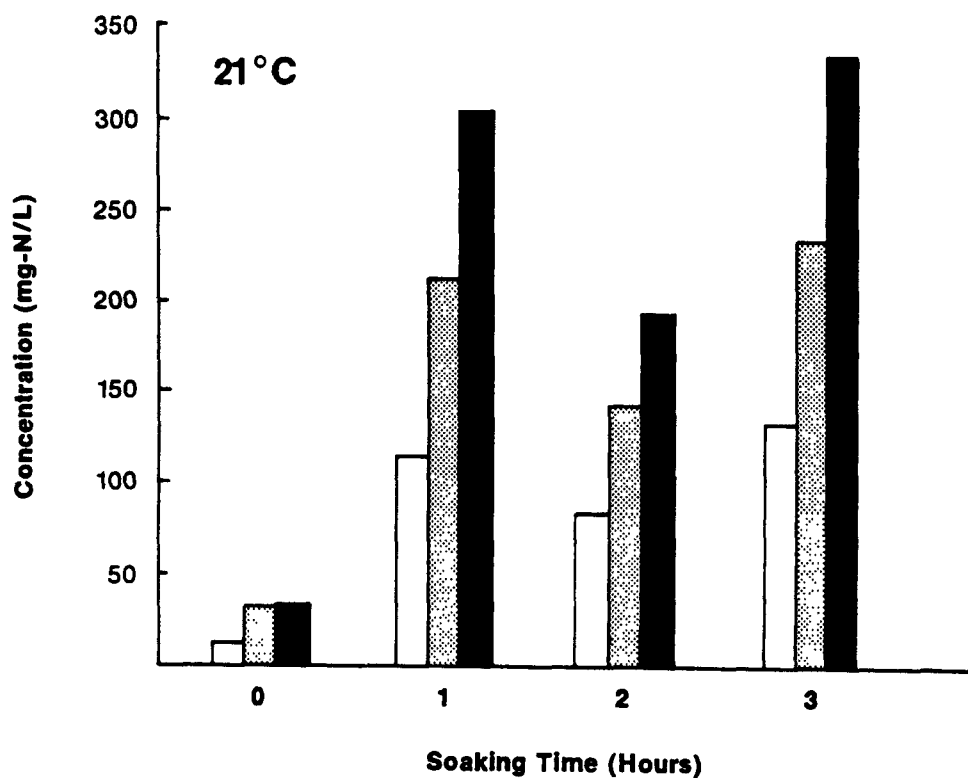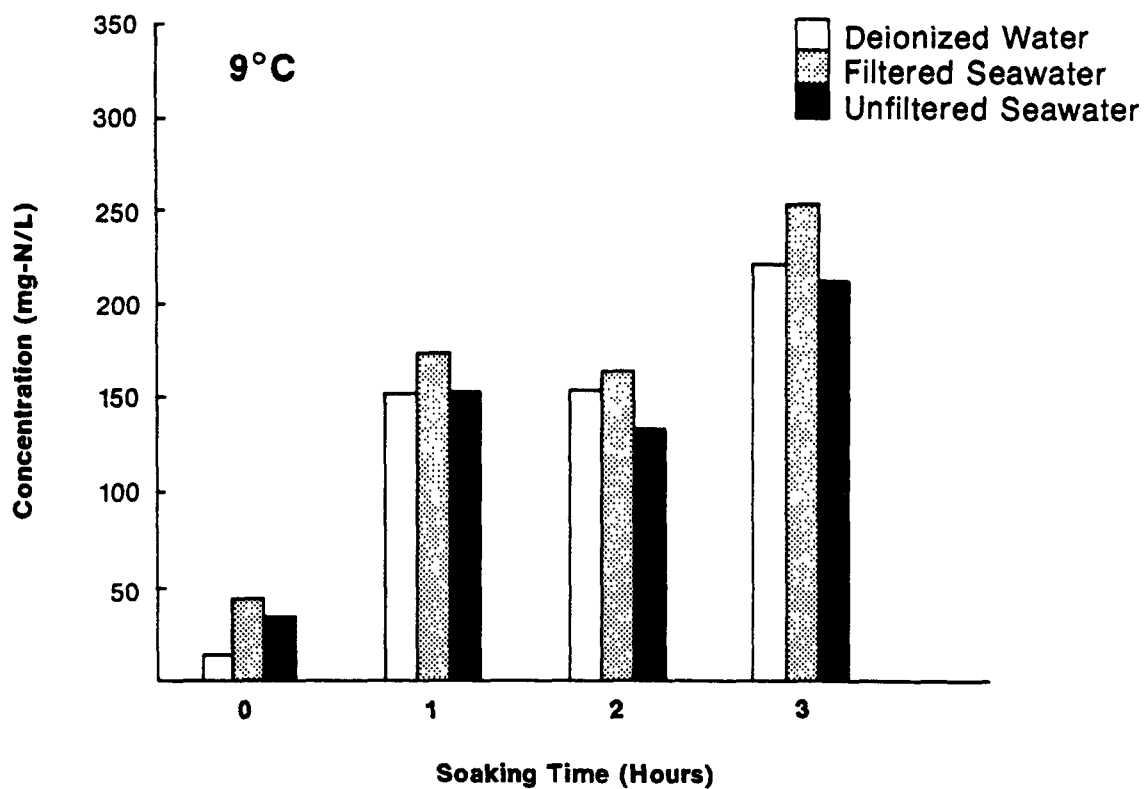
Figure 2.4. Ammonia Release From IBDU Briquettes at 9 and 21 Degrees Centigrade In 3 Different Water Sources.

PAR EX Granules

A typical cumulative nutrient release pattern for ammonia, phosphate, and TKN from PAR EX granules in bags from one of the static tests is shown in Figure 2.5. High amounts of nutrients were released initially, followed by a very slow release. If the experiment was repeated with the granules layered on the bottom of the beaker (i.e., no bag to contain the granules), a steeper release pattern was observed. The number of water exchanges had a much greater effect on these experiments than when the fertilizers were placed in bags (data not shown).
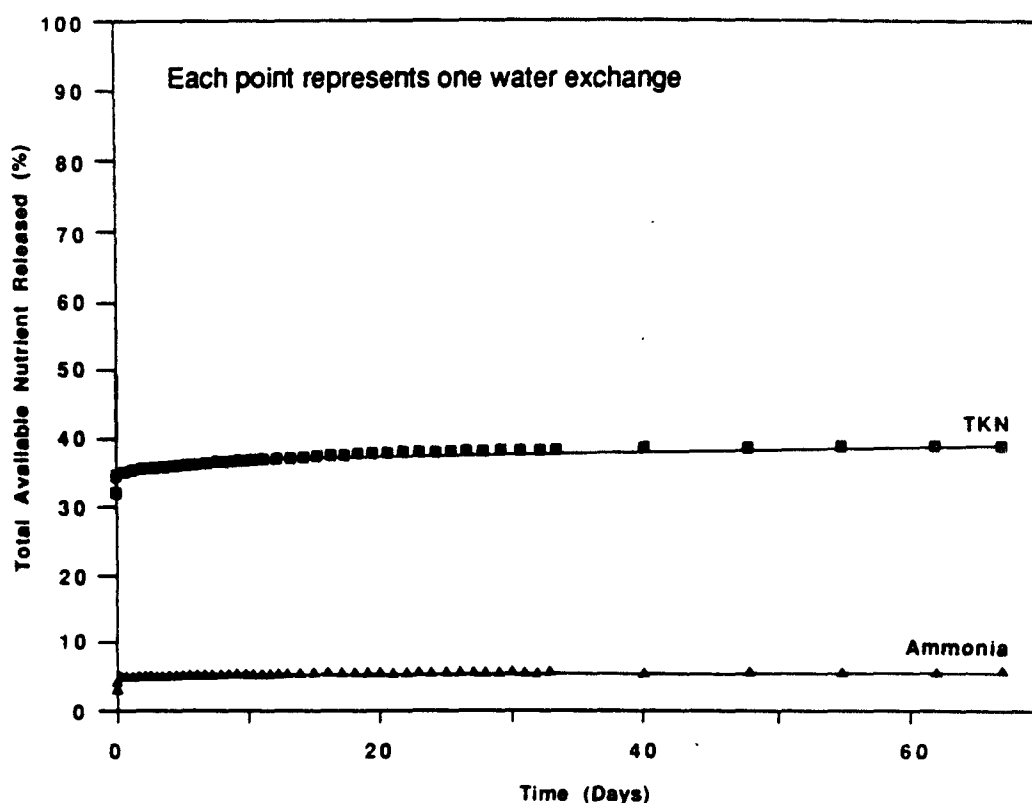
Figure 2.5. Cumulative Release of Ammonia and Total Kjeldahl Nitrogen (TKN) from IBDU Fertilizer Granules Contained In Bags In Static Flask Experiments.

Additional studies also showed that if the granule bag volume is reduced relative to the bag surface area, slightly more nutrients were released (Table 2.2). Thus, the more water passing over the granules, the higher the release rate. As expected, the results of the test using Prince William Sound beach material were similar to the test without rocks.

TABLE 2.2. TOTAL KJELDAHL NITROGEN (TKN) RELEASED UNDER STATIC
CONDITIONS FROM IBDU GRANULAR FERTILIZER IN BAGS WITH DIFFERENT
SURFACE TO VOLUME RATIOS

Bag Volume/Surface Area (cubic cm/square cm)	Fertilizer Weight (g)	Seawater Volume (ml)	% of Cumulative Available Nitrogen (TKN) Released in:	
			24 Hours	45 Days
1.8	893	4,800	34	38
1.3	256	1,400	36	42
0.6	32	450	41	49

OSMOCOTE Briquettes

The cumulative nutrient release pattern for ammonia, phosphate, and TKN for static tests is shown in Figure 2.6. After 2 months of testing, approximately 25% of the available nitrogen was released, primarily as TKN, whereas almost 60% of the phosphate was released over this time period. The physical form of the briquette was unstable, flaking soon after initial submersion and further decomposing over time. A dye within the briquette turned the water green with each water exchange. These briquettes, despite their good nutrient release characteristics, appeared unsuitable for long-term use in the field.

MAGAMP Briquettes

When high-density and low-density MAGAMP briquettes were tested, the low-density briquette (about half the weight of the high density) disintegrated almost immediately upon submersion in the seawater, and consequently was not tested. Release of ammonia, TKN, and phosphate from the high-density briquettes was slow and constant (Figure 2.7). After 10 days, only 1.5% of the available nitrogen was released. At 75 days, approximately 5% to 6% had been released. The release rate appeared to be independent of the number and volume of water exchanges. The high-density briquettes appeared to be very durable. When MAGAMP powder was tested, it congealed to a putty-like consistency soon after the experiment was started. Accumulative release of ammonia was about the same as the briquettes.
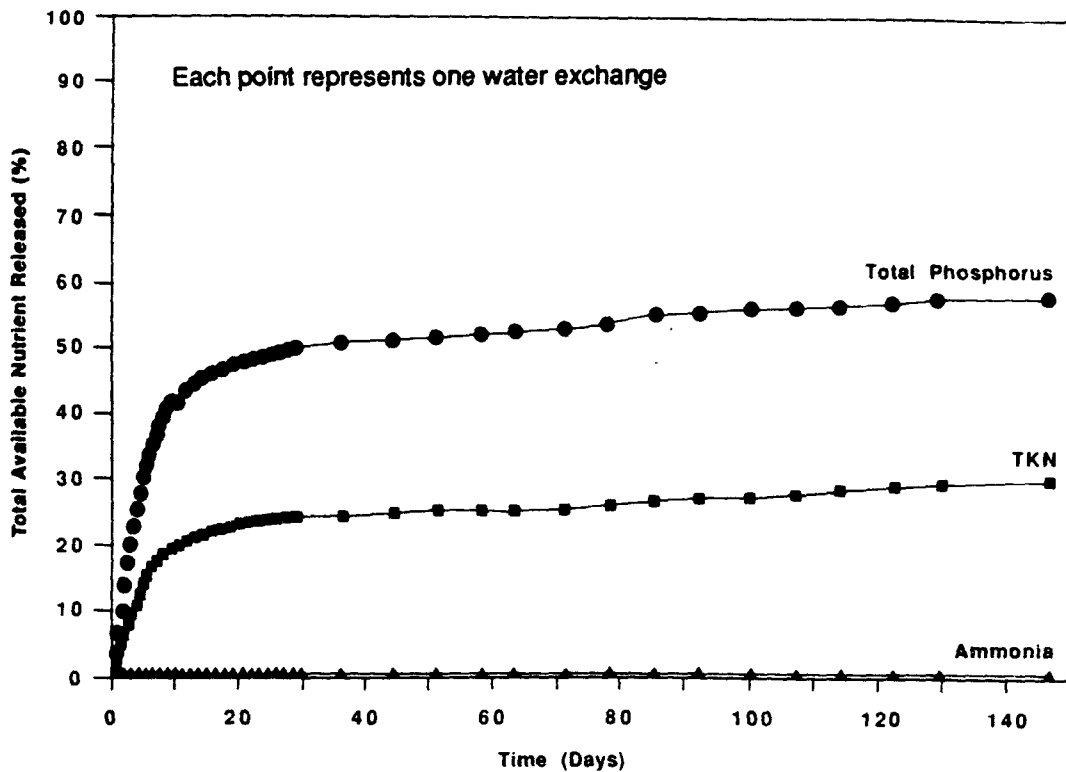
Figure 2.6. Cumulative Release of Ammonia, Total Kjeldahl Nitrogen (TKN), and Total Phosphorus from OSMOCOTE Briquettes in Static Flask Experiments.
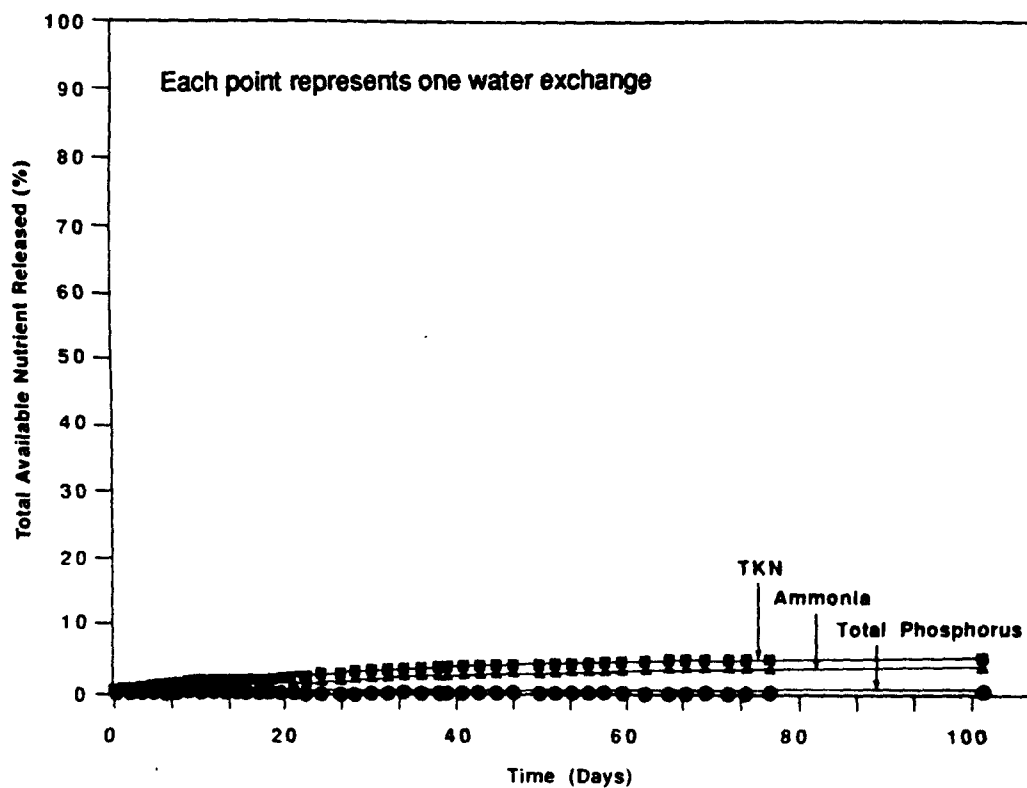
Figure 2.7. Cumulative Release of Ammonia, Total Kjeldahl Nitrogen (TKN), and Total Phosphorus from MAGAMP Briquettes in Static Flask Experiments.

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Results from the intermittent submersion tests showed the same low release rates. A high burst of ammonia seen within the first half hour of the test was explained by initial flaking of the briquettes. Flaking at later times did not occur.

MAGAMP can be formulated into dense bricks. Bricks weighing 8 and 40 lbs were field tested as an alternative physical form for fertilizer application. These bricks are useful in the field because of their positional stability on the beach without an anchoring device. However, these bricks could not be produced in large quantity and were, therefore, unavailable for use in any of the fertilization studies. However, because of their potential promise as an alternative physical form of fertilizer, separate beach mechanics studies were conducted to evaluate the nutrient release and distribution characteristics of these bricks. The very slow release of ammonia from MAGAMP made it necessary to determine, under controlled field conditions, if nutrient release could be detected in the field.

Beach pore water sampling stations were placed down-gradient from MAGAMP bricks as shown in Figure 2.8. Samples for ammonia analysis were collected 12, 24, and 96 hours after initial placement of the bricks. The data are shown in Figure 2.9. The 40 lb brick released up to 138 μ of nitrogen as ammonia, with the highest concentrations directly down-gradient from the block. Significant quantities of ammonia were observed up to 2 m from the bricks at low tide. Ammonia also appears to be well distributed around the brick. The data suggest that this type of point source for fertilizer application could be quite useful in the future.

SIERRA CHEMICAL Granules

Figure 2.10 shows the cumulative nutrient release pattern with a variable exchange rate (5 exchanges on the first day, 2 exchanges per day thereafter through the 10th day, daily thereafter through the 40th day, and then every other day. The amount of nitrogen (ammonia and nitrate) released after 80 days was 77% of the total available nitrogen. When the frequency of water exchanges was doubled, 95% of the total nitrogen (approximately half ammonia and half nitrate) was released after 80 days. The shape of the release curves were similar. This effect of water exchanges was probably due to the mechanical agitation of the system prior to each exchange.

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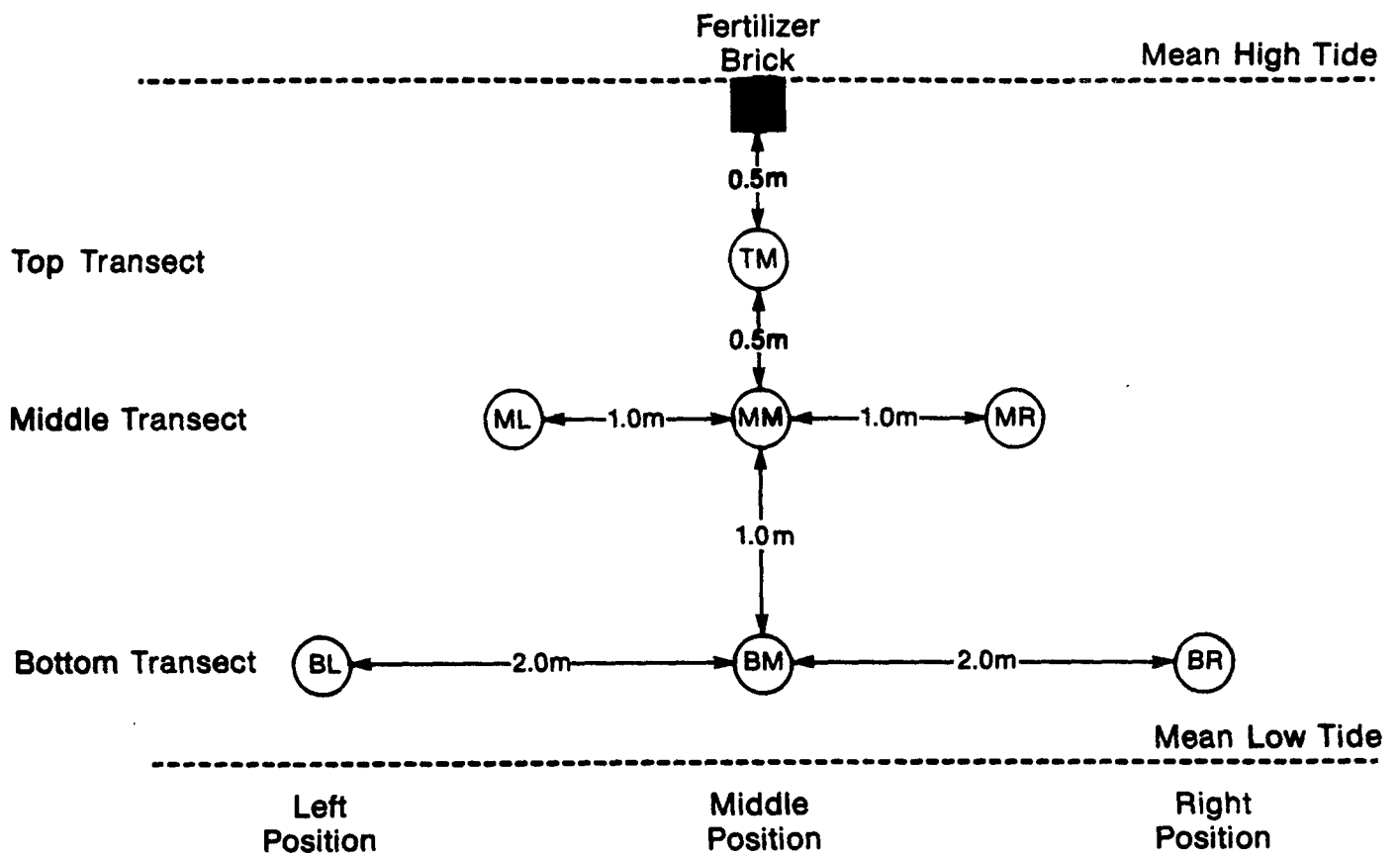
Figure 2.8. Sampling Point Locations for Magnesium Ammonium Phosphate Fertilizer Field Test.
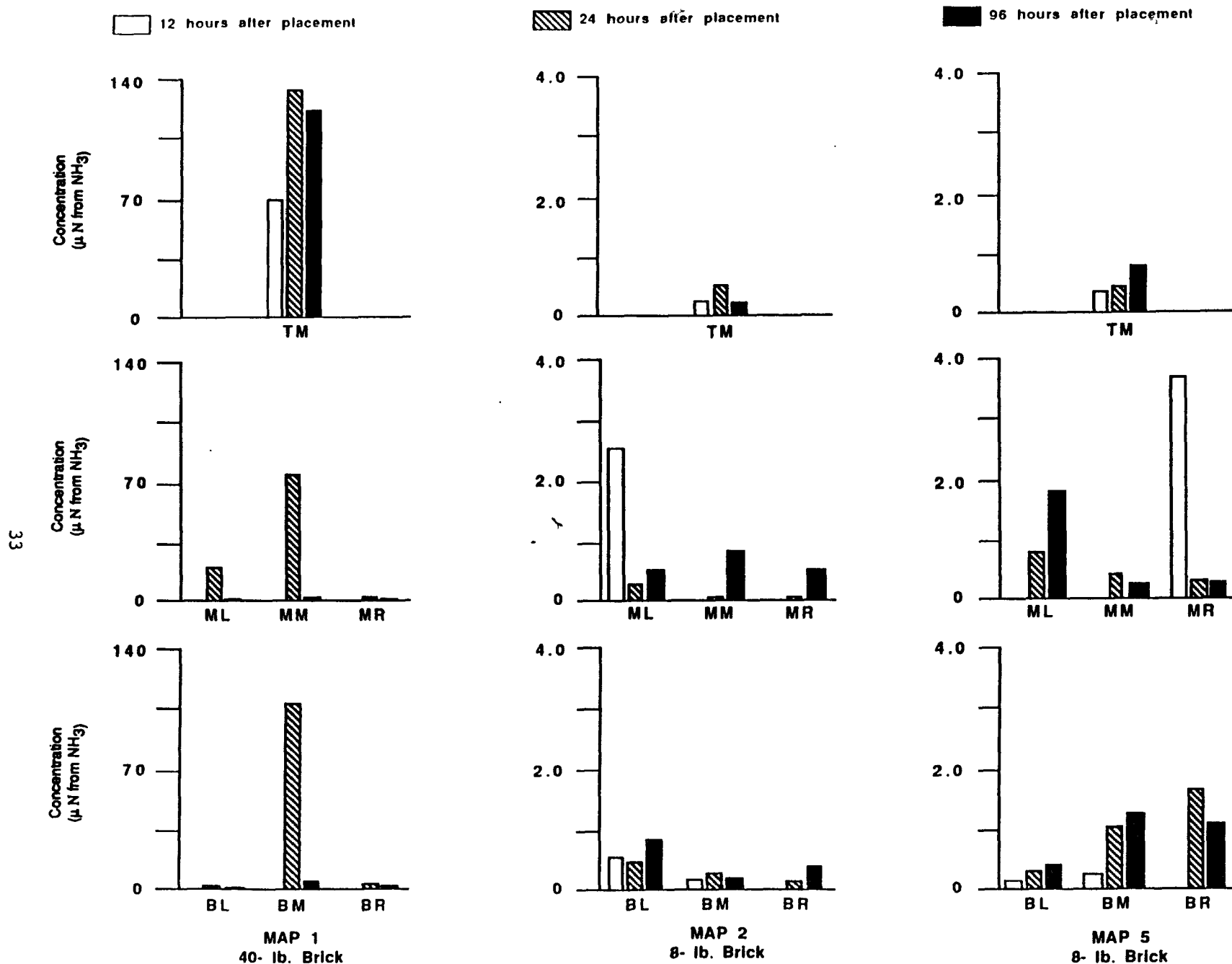
Figure 2.9. Magnesium Ammonium Phosphate Fertilizer Test: Ammonium Concentration in Beach Pore Water at 12, 24 and 96 Hours After Placement of Fertilizer (Sampling Locations Given in Figure 2.8.).
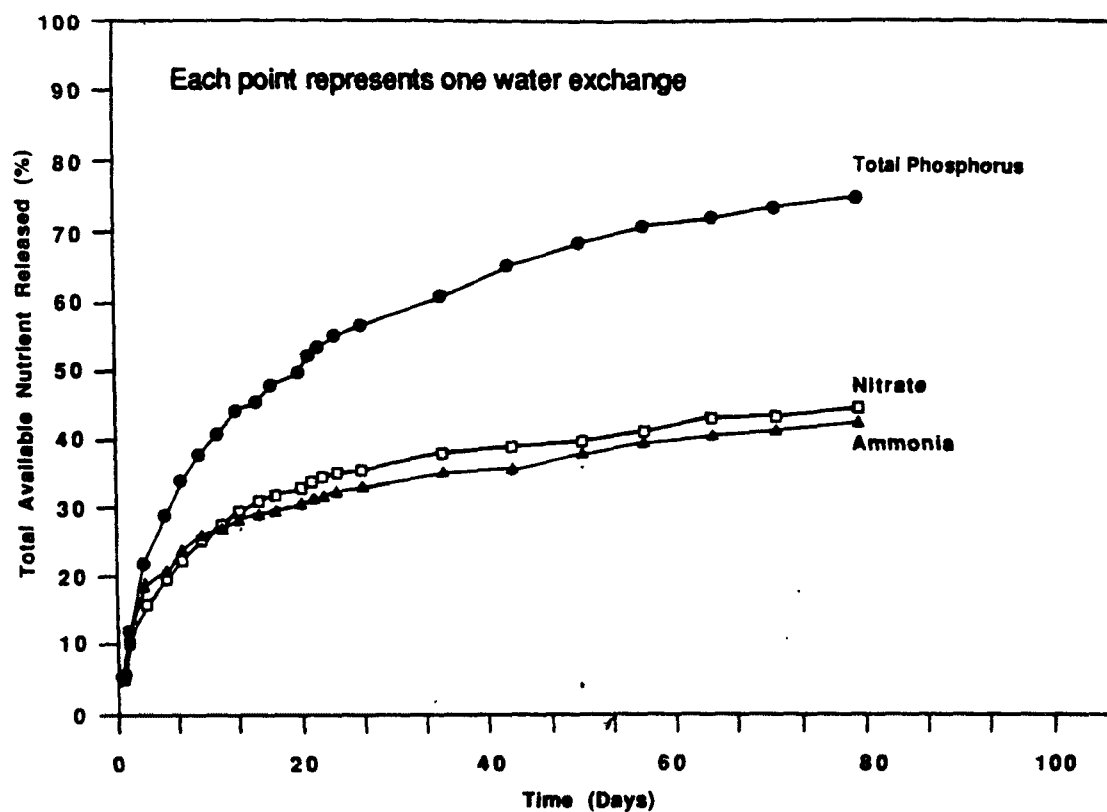
Figure 2.10. Cumulative Release of Ammonia and Nitrate from SIERRA CHEMICAL Granules in Static Flask Experiments.

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INIPOL Oleophilic Fertilizer

The results from static tests with this fertilizer are shown in Figure 2.11. All of the nitrogen (>100%) was released within the first few water exchanges. The release of more nitrogen than was theoretically thought to be in the INIPOL formulation suggests that manufacturer's specifications for this batch of INIPOL were incorrect or TKN was present on the oiled beach material.

An intermittent submersion test was conducted on the oleophilic fertilizer applied to oil-covered Prince William Sound beach material. The data are shown in Table 2.3. Within 5 minutes after INIPOL-treated oiled rocks were covered with seawater, over 60% of the available nitrogen was released as TKN. However, following this initial burst, TKN appeared to be released more slowly (i.e., very little increase in TKN occurred over the next 115 minutes). After decanting the water off the beach material, allowing it to sit unsubmerged for 6 hours and recovering the rocks with water, only 8.3% of the available nitrogen was further released as TKN. Concentrations of ammonia and phosphate released were quite low, but generally followed the same pattern as the TKN.

Allowing the fertilizer to remain in contact with the oil for 6 hours prior to the addition of water did not change the nutrient release patterns. This suggests that the amounts of nutrient which sequester with the oil (i.e., not washed off) are incorporated very soon after fertilizer application. It is unclear, however, why the nutrient did not sequester with the oil in the static experiments.

In addition, mixing the beach material as the INIPOL was applied, or warming the INIPOL to 25°C before application, did not significantly change the amount of nitrogen released in the first few minutes. It is unclear why the static tests did not show similar results.

SUMMARY AND CONCLUSIONS

From these studies, three fertilizer formulations were selected for field testing: the WOODACE slow-release fertilizer briquette formulation, the CUSTOMBLEN fertilizer granule formulation, and the INIPOL oleophilic fertilizer formulation.

It was concluded that bags of WOODACE fertilizer briquettes would be used in the initial field demonstration for slow-release fertilizer. This fertilizer had good nutrient release characteristics, excellent durability in the field, and ready availability. Given the time constraints of the bioremediation field demonstration project, this fertilizer was a reasonable first choice.
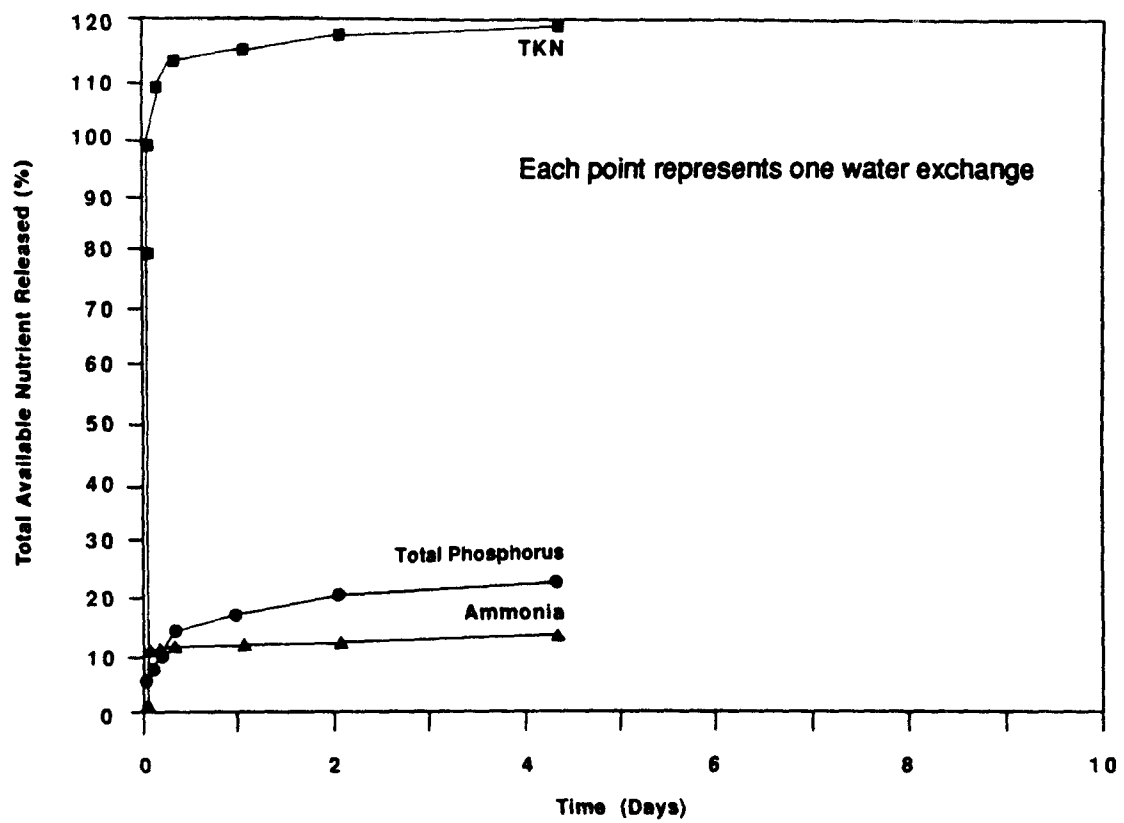
Figure 2.11. Cumulative Release of Ammonia and Total Kjeldahl Nitrogen (TKN) from INIPOL EAP 22 in Static Flask Experiments.

TABLE 2.3. RELEASE OF AMMONIA, TOTAL KJELDAHL NITROGEN (TKN), AND
TOTAL PHOSPHORUS (TP) FROM INIPOL EAP 22 DURING INTERMITTENT
SUBMERSION EXPERIMENT

Minutes from Start of Experiment	5 Minute Contact Time[a]	6 Hour Contact Time[a]
Ammonia Released (mg N/L)[b]		
5	1.1	0.5
15	1.1	0.4
30	1.4	0.5
60	1.3	0.7
120	1.4	0.7
510[c]	0.2	
540	0.1	
600	0.0	
TP Released (mg P/L)		
5	1.3	1.4
15	1.2	1.2
30	1.0	1.1
60	1.5	1.3
120	1.0	1.0
510[c]	1.1	
540	0.9	
600	0.9	
TKN Released (mg N/L)[b]		
5	24.6	29.8
15	26.1	34.8
30	27.2	35.5
60	32.5	34.3
120	29.4	32.3
510[c]	4.6	
540	4.6	
600	4.3	

[a] Time between fertilizer application and initial submersion
[b] Initial concentration of nitrogen = 57 mg/L
[c] Water drained; beach material remained unsubmerged for 6 hours; seawater replaced

Recognizing that bagged briquettes could not be produced in sufficient quantities for large-scale application, slow-release fertilizer granules (SIERRA CHEMICAL, CUSTOMBLEN) were selected for the second field test, since they could be easily broadcast over the beach surface in a large-scale operation. The granules had good nutrient release characteristics but were not as long lasting or as durable as the briquettes.

Tests with the oleophilic fertilizer, INIPOL EAP 22, showed that it did not retain nutrients on the surface of oil, losing approximately 90% to 100% of the available nitrogen in the first minutes following application. However, more elaborate microcosm studies using more realistic environmental conditions (Roger Prince, Jim Bragg, Exxon) have shown that only approximately 50% of the nitrogen is lost in the first 24 hours and small amounts are released thereafter for 2 to 3 weeks. Other published studies on this oleophilic fertilizer have shown it to work well on sandy beaches, but similar testing has not been done with cobble beaches. Therefore, its use on the cobble beaches found in Prince William Sound represented a new application.

SECTION 3
TEST PLOT DESIGN AND SAMPLING

STUDY AREA DESCRIPTIONS

Beaches in Snug Harbor and Passage Cove were selected in 1989 as test sites for the application of the two slow-release fertilizers (WOODACE briquettes and CUSTOMBLEN granules), and the oleophilic fertilizer (INIPOL). In addition, the application of a fertilizer solution was also tested at the Passage Cove site.

Selection of these test sites was based on the following criteria:

● Typical shoreline of Prince William Sound; i.e., cobblestone overlain on mixed sand and gravel beaches with a gradual vertical rise

● Sufficient area for testing with fairly uniform distribution of beach material

● Protected embayment with adequate staging areas

● Uniform oil contamination

● Minimal impact from freshwater inputs

In the summer of 1990, beach areas at Disk Island were selected as test sites for application of CUSTOMBLEN granules, and beach areas at Elrington Island were selected for the application of nutrient solution.

Snug Harbor

Snug Harbor is located on the southeastern side of Knight Island. The shoreline utilized for the demonstration is located on the western side of this harbor (Figure 3.1). This shoreline represented a beach with oil contamination that approximated the degree of contamination remaining after a moderately oiled beach had been physically washed by the Exxon process. This approximation was required because at this time there were no beaches available for testing in Prince William Sound that had been physically washed. The area is surrounded by mountains, reaching an elevation of approximately 2,000 feet, with steep vertical ascents. Major sources of freshwater runoff are from precipitation and snowmelt, which is typical of islands in Prince William Sound. Although other

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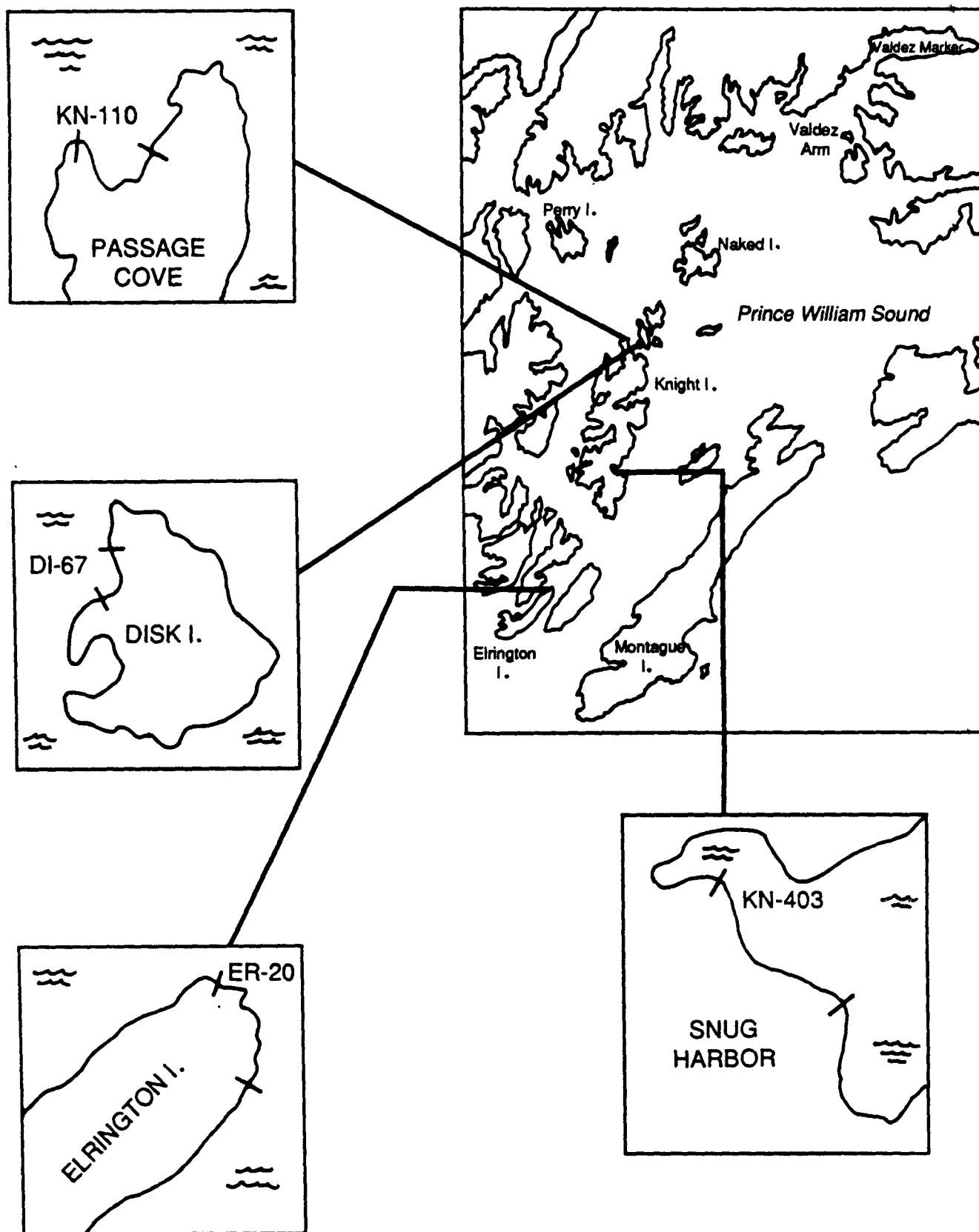
3.1. Location of Field Sites. Alphanumeric Codes Were Those Designated by Exxon and the Prince William Sound Shoreline Cleanup Committee.

shorelines in Snug Harbor were heavily contaminated with oil, it appeared that little oil was being released to the water, thus minimizing the prospect of reoiling the beaches chosen as control and treatment and control. Table 3.1 identifies the beach types and dimensions.

TABLE 3.1. PHYSICAL DESCRIPTION OF TREATMENT AREAS AT SNUG HARBOR

Beach	Beach Type	Length (m)	Depth (m)
Eagle	Sand, gravel	21	12
Otter	Sand, gravel	21	12
Otter	Sand, gravel	35	12
Seal	Cobble	28	12
Seal	Cobble	28	12
Seal	Cobble	21	8

Oil contamination in the test area was present as a continuous band along the length of the beach. This band was approximately 15 to 20 meters wide and corresponded roughly with the average boundaries of the high and low tides observed in Snug Harbor. To determine the approximate distribution of oil on the beach, samples of beach material from one of the designated mixed sand and gravel plots were taken on May 25, 1989. The samples were extracted, and the oil weight and chemical composition were determined. Methods for the sampling and analysis are given in Section 4. The oil residue weights and ratios of nC17/pristane and nC18/phytane at two different depths are shown in Table 3.2. It is clear that oil concentrations varied considerably, ranging from a high of 67,200 mg/kg of beach material to a low of 8 mg/kg of beach material. In general, higher concentrations were found in the top 10 cm of the beach. Changes in the ratios, relative to fresh Prudhoe Bay crude oil, were also apparent in some samples, indicating biodegradation of the oil. Changes were quite variable, but it does appear that biodegradation may have been occurring at the lower depths.

TABLE 3.2. ANALYSIS OF OIL EXTRACTED FROM MIXED SAND AND GRAVEL SAMPLES TAKEN FROM OTTER BEACH IN SNUG HARBOR ON MAY 28, 1989, TWO WEEKS PRIOR TO FERTILIZER APPLICATION[a]

Block No.	Top (0-10 cm)			Bottom (10-20 cm)		
	Residue wt. (mg/kg)	nC17/ Pristane[b]	nC18/ Phytane[b]	Residue wt. (mg/kg)	nC17/ Pristane	nC18/ Phytane
1	100	0.8	1.0	253	0.9	0.8
2	29,000	1.6	1.9	18,300	1.6	2.0
	30,100	1.5	1.7			
3				296	1.0	1.2
4	6,070	1.5	1.8	2,600	1.5	1.9
5	2,030	1.2	1.5	37	0.8	1.0
6	6,600	1.2	1.7			
7	1,440	1.1	1.4			
8	1,030	0.8	1.1			
9	7,600	1.4	1.7			
10				97	1.1	1.3
				365	0.8	1.1
				469	0.9	1.1
				412	0.9	1.1
11	9,820	1.5	1.8	512	1.2	1.5
12	658	1.5	1.9	8	1.0	1.2
13	67,200	1.6	1.8	9,280	1.6	1.8
				9,620	1.5	1.8
				8,100	1.6	1.9
14				45	0.9	0.9
15				538	1.3	1.6
16				80	0.9	1.3
17						
18				622	1.1	1.4
19				125	0.9	1.3
20	1,560	1.0	1.3			
21	8,190	1.6	1.7	1,790	1.4	1.6
Mean	12,242	1.3	1.6	2,169	1.1	1.4
Std Dev	+/-18,556	+/-0.3	+/-0.3	+/-4,842	+/-0.3	+/-0.3

[a] Otter Beach was divided into three equal zones lengthwise across the beach to represent high, mid, and low tide areas. Each zone was divided into 7 equal blocks, and blocks were numbered from left to right consecutively, starting with the high tide zone.

[b] nC17/pristane and nC18/phytane ratios in fresh Prudhoe Bay crude oil are approximately 1.7 and 2.0, respectively.

Passage Cove

Passage Cove is located on the northwestern side of Knight Island (Figure 3.1). This site was originally heavily contaminated with oil and was subjected to physical washing by Exxon. Even after physical washing, considerable amounts of oil remained at this site, mostly spread uniformly over the surface of rocks and in the beach subsurface. Pools of oil and mousse-like material were minimal on the surface. Contamination was apparent to approximately 50 cm below the beach surface. All beach areas tested were cobblestone set on a mixed sand and gravel base. Table 3.3 lists Passage Cove beach designations and plot dimensions. This site served as the main reference beach for the large-scale application of fertilizers and was used to evaluate the application of fertilizer solutions.

TABLE 3.3. PHYSICAL DESCRIPTION OF TREATMENT AREAS AT PASSAGE COVE

Beach	Beach Type	Length (m)	Depth (m)
Raven	Cobble over mixed sand and gravel	28	21
Tern	Cobble over mixed sand and gravel	35	21
Kittiwake	Cobble over mixed sand and gravel	28	21

Disk Island

Disk Island is a small island located between Ingot and Knight Islands. Study site DI-067a is located on the upper northwest corner of Disk Island (Figure 3.1). The site was moderately oiled and not subjected to physical washing by Exxon. The beach was a fairly wide, low energy, cobble beach with both surface and subsurface contamination. The test plots within the beach were located near a pond, and large trees lined the back of the beach. In addition to the experiments conducted by EPA's Bioremediation Project at Disk Island, experiments were also conducted at the same site by EPA/ Cincinnati utilizing commercial microbial products. This study is discussed in Section 13.

Elrington Island

Elrington Island was the southernmost test area utilized by the oil spill project, and is located between Latouche and Evans Islands. Test site section ER-20 on the north end of Elrington Island represented a narrow, cobble, high-energy beach with typical subsurface oil contamination (Figure 3.1). A berm separated the beach from the treeline. The surface of the beach was relatively free of oil but extensive amounts of oil were found approximately 15 to 30.5 cm below the surface. This section was therefore an appropriate beach to determine the effectiveness of fertilizer solutions in enhancing biodegradation of the oil in the subsurface.

SAMPLING METHODS IN 1989

Snug Harbor

The beach sampling design was formulated to generate scientifically defensible conclusions relative to the success of bioremediation (Figures 3.2 and 3.3). Unless otherwise specified, each test site was divided into a series of plots within the beach. The plots were generally 21 to 35 m long and 12 m wide running the length of the beach. Plot size was controlled by the available beach (i.e., sections of relative uniformity), the extent of beach covered by the oil, and the prominence of certain topographical features. Buffer zones of at least 5 m separated the plots. Larger buffer zones (>20 m) were established between treated and reference plots to minimize cross-contamination. Cross-contamination of nutrients between plots was not expected because of extensive dilution.

Zonal sampling (low, mid, and high tide) was used to uncover any effect due to seawater coverage, rainfall, and freshwater runoff or temperature (exposure to sun, air, ocean, etc.). Sampling intensity was gauged to minimize biological and physical effects. Sampling was designed to permit collection of a sufficient number of samples to establish active biodegradation in each zone. If degradation occurred in all three zones, comparisons could be made to establish trends from high to low tide zones.

Blocks were derived from each intertidal zone by dividing the beach plot length into seven equal segments (Figure 3.4). For three zones that created a total of 21 blocks. It was recognized that certain sampling points on the beach were not representative of the entire beach. For instance, stream runoff over one section of the beach might have been caused by an underlying solid rock outcrop near the
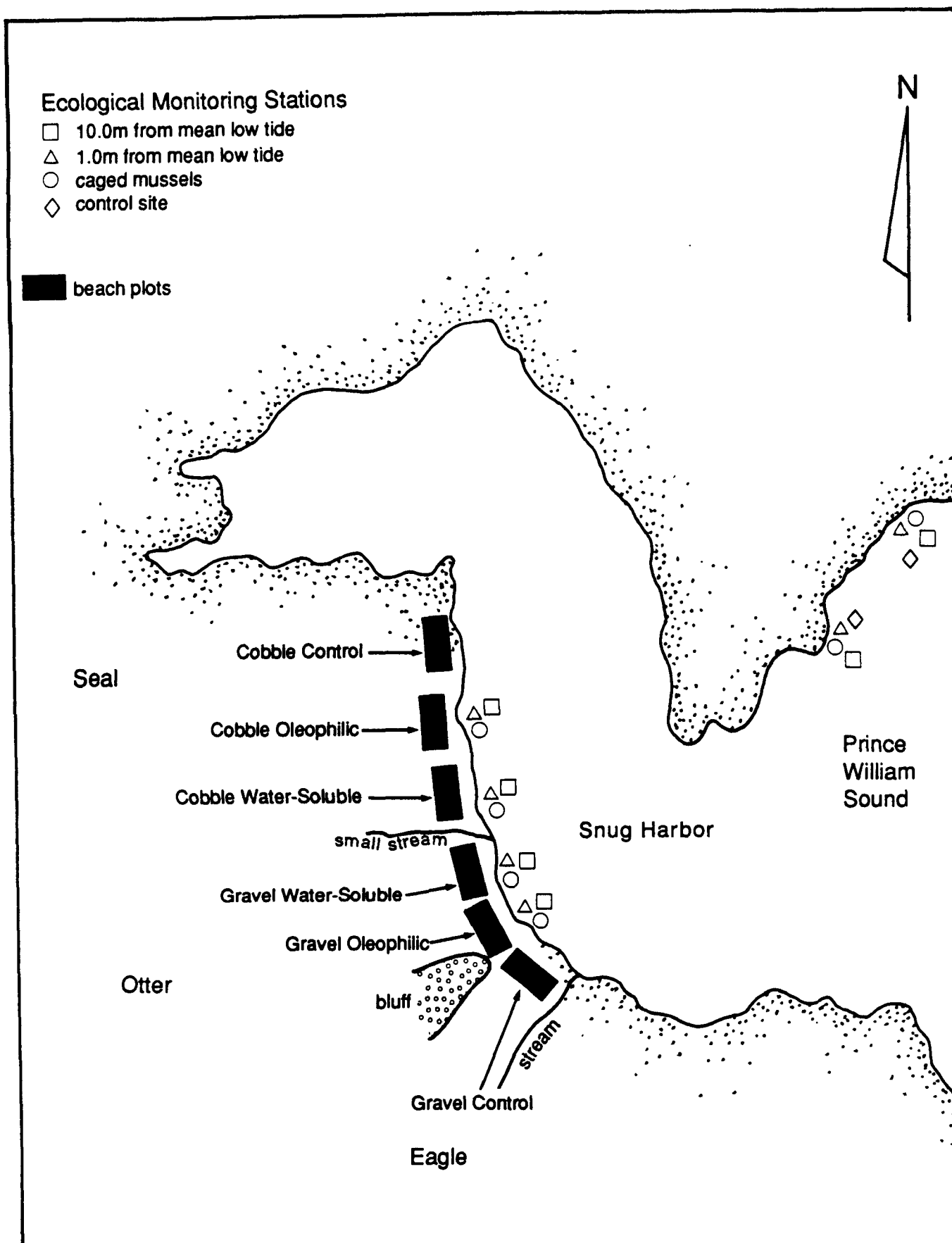
Figure 3.2. Sampling Locations at Snug Harbor, Knight Island, In Prince William Sound, Alaska.
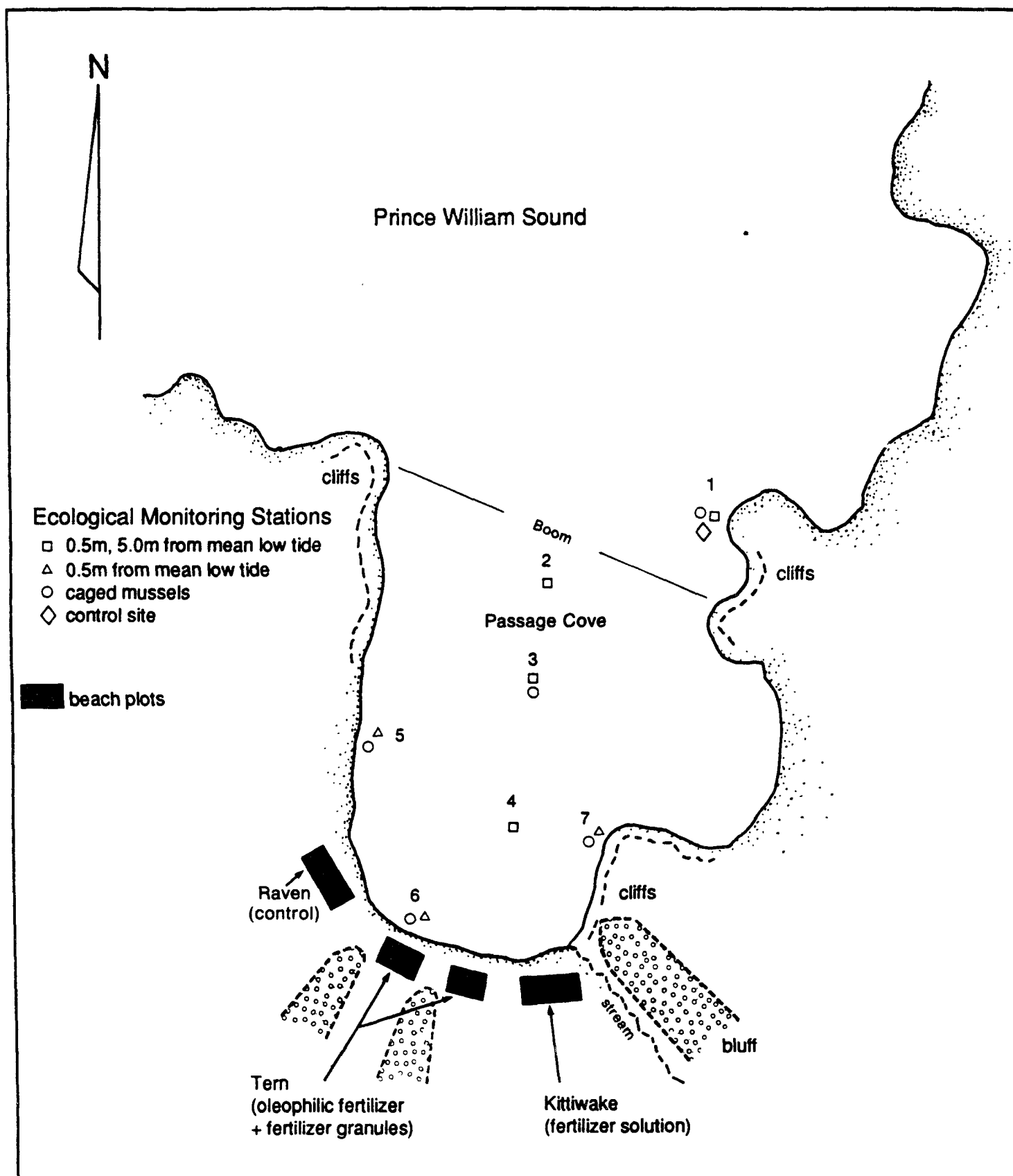
Figure 3.3   Sampling Locations at Passage Cove, Knight Island, in Prince William Sound, Alaska.
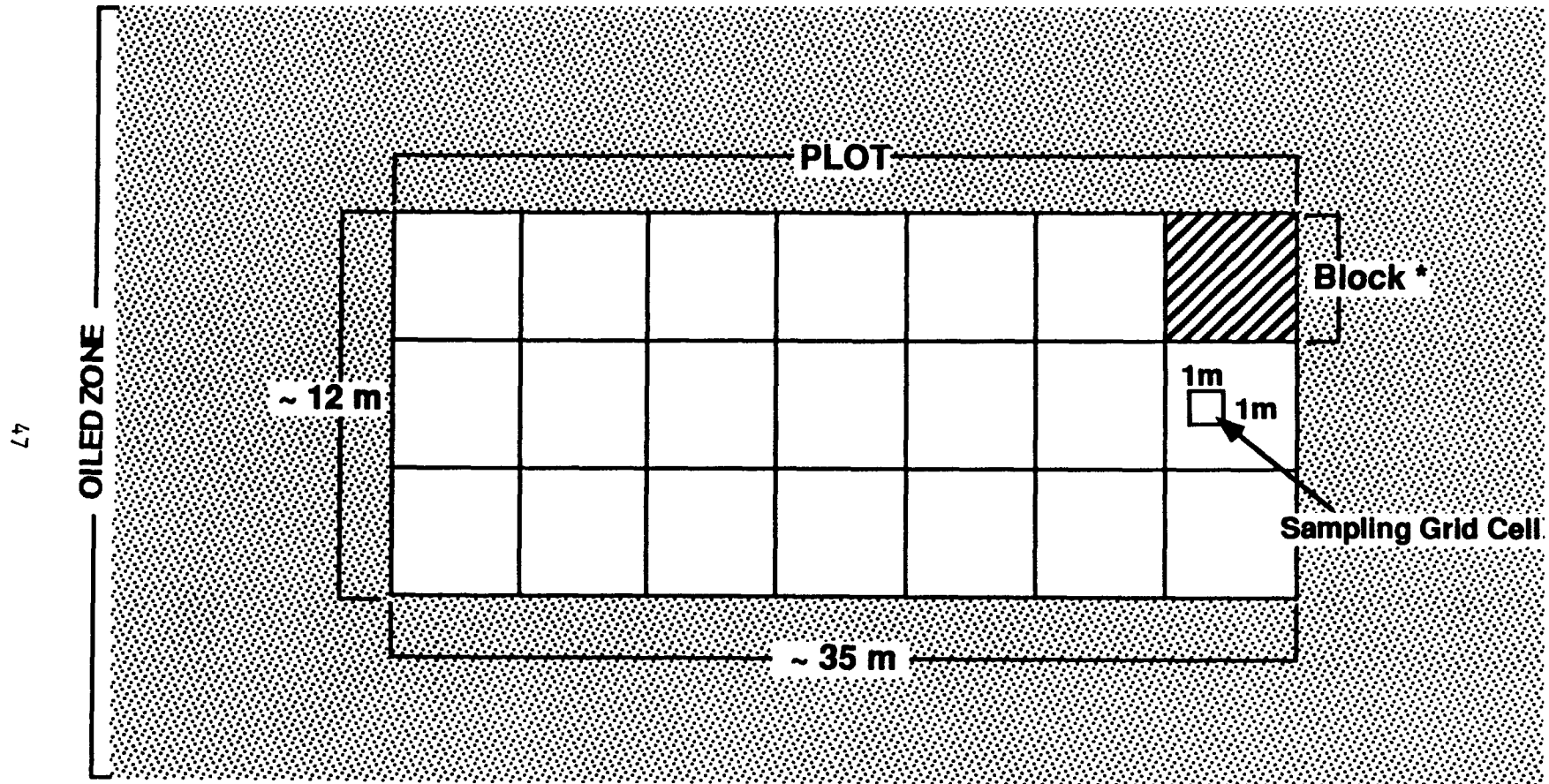
Figure 3.4. Sampling Design for Snug Harbor and Passage Cove

* Each group of seven blocks represents an intertidal zone (high, mid, and low)

surface of the beach. Collecting seven samples for each beach stratum accounted for collection of a nonrepresentative sample, and for the possibility of obvious gross error in a sample due to incorrect sampling or analysis. In addition, seven samples were needed to ensure adequate power of statistical tests.

Each block was sampled within 1 m x 1 m sampling grid cells. Therefore, although the number of blocks within plots did not vary with beach size, the number of sampling grid cells within a block for a particular plot did. The boundaries of each plot were established using rope secured to rebar stakes. Squares of PVC pipe (1 m x 1 m) were used to delineate sampling grid cells.

For each designated sampling time, a sample was taken from one grid cell within each block for all anticipated analyses. The sampling grid cell selection procedure used the following steps:

- The sampling crew began at the upper-left-hand corner of each block and picked two numbers from a random number table that fell within the confines of the block. For example, if the block size for the particular plot was 5 m in length and 3 m in width, the table was used to choose a number from 1 to 5 to designate the distance along the block boundary where the grid cell would be established from the starting point. The intersection of the two randomly selected points was the upper-left-hand corner of the selected sampling grid cell. The same sampling grid cell location was used for all blocks in a single plot during a single sampling event.

- A 1 m x 1 m frame was placed on the beach in the designated grid cell and samples were collected from the center of the frame (Figure 3.5)

- If a sample could not be taken at the center of the grid cell, a random number between 1 and 12 was chosen. These numbers represented positions on the face of a clock, in which 12 pointed to high tide line. The sampler then moved away from the center of the frame toward the indicated clock position until an appropriate site was found within the sampling frame. The sampling crew used judgment in many situations; for example, if a large boulder was encountered, the site was discarded and the selection procedure was repeated.

This procedure was continued for each block until sampling was completed. Except for nutrients, sampling site selection was the same for all sample analyses. All sampling was performed at low tide. One to two days were required to sample all plots at each test site depending on the height of low tide. When two days were required for sampling, only one-half of the untreated control plots were sampled each day.

Figure 3.5. A 1m by 1m Frame Was Placed on the Beach in the Designated Grid Cell and Samples Were Collected from the Center of the Frame.

The overall design of beach sampling efforts was statistically non-optimal. The major limitation arose from the lack of duplicate beaches for each treatment and reference site. Measured effects were attributable to both nutrient treatment effects and beach effects. It could not be determined statistically whether an increased bioremediation rate at a site was due to either the treatment or to a fortuitously good location, since these two variables were confounded. When only one treated beach was successful, low confidence should be assigned to the result; however, because two types of beaches and two types of treatments were used, when one or both treatments were successful on both types of beaches, confidence in the results may be high.

Samples were taken on mixed sand and gravel beaches by placing a bottomless metal pail onto the beach surface and working the bucket into the substratum. As small rocks were encountered that prevented the pail from going further into the beach material, the material around the pail was

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excavated and the rock removed. If 50% of the rock was inside the perimeter of the pail, it was added to the pail and included in the sample. If 50% or more was on the outside, it was excluded from the sample. All large rocks (approximately 4 cm or larger in any dimension) were discarded from the sample, since the amount of oil covering their surface was insignificant relative to oil in the entire sample, and exclusion of these rocks reduced variability in substrate characteristics of the sample.

Once the pail was inserted approximately 13 to 14 cm into the beach material (using marks on the inside of the pail), all beach material to 10 cm (including small rocks that protruded more than 50% above that mark), were included in the sample. Rocks that did not protrude more than 50% above the mark were not included. All beach material removed from the sampler was placed in washed and rinsed new paint cans. The contents of the paint can were then thoroughly mixed with a steel spoon. A subsample of material sufficient to fill a 400 ml wide-mouth jar was taken from the mixed sample. The jar and its contents were also subsampled for microbiology analysis and then frozen.

Cobblestone beaches were sampled in a similar manner. The bottomless pail was worked down over the cobble to the surface of the mixed sand and gravel layer under the cobble. Approximately 5 to 10 cobblestones were then sampled at random without regard to the extent of their oiling, and the cobblestones were placed on large squares of aluminum foil. The stones were then double-wrapped with the foil and placed in a ziploc plastic bag. The package was then frozen. Cobblestones remaining in the bottomless pail were removed and the mixed sand and gravel sampled as above.

Passage Cove

The methods for test plot design and sampling for Passage Cove were identical to Snug Harbor, but different plot sizes were delineated.

SAMPLING METHODS IN 1990

Sampling baskets (16 cm x 16 cm x 16 cm wire mesh containers with lids) containing homogenized oiled beach material were prepared in the field for Disk Island and Elrington Island (Figure 3.6). Oiled rock material from Prince William Sound beaches were sieved for material within 12.5 mm, and then manually thoroughly homogenized. The homogenized material was then placed into each basket. Two types of baskets were created: one filled completely with homogenized oiled

Beach Surface

Wire Mesh Top

Un-Oiled Beach Material — 4 cm

Subsurface Oil

Oiled Beach Material — 8 cm

Subsurface Oil

Un-Oiled Beach Material — 4 cm

Wire Mesh Basket
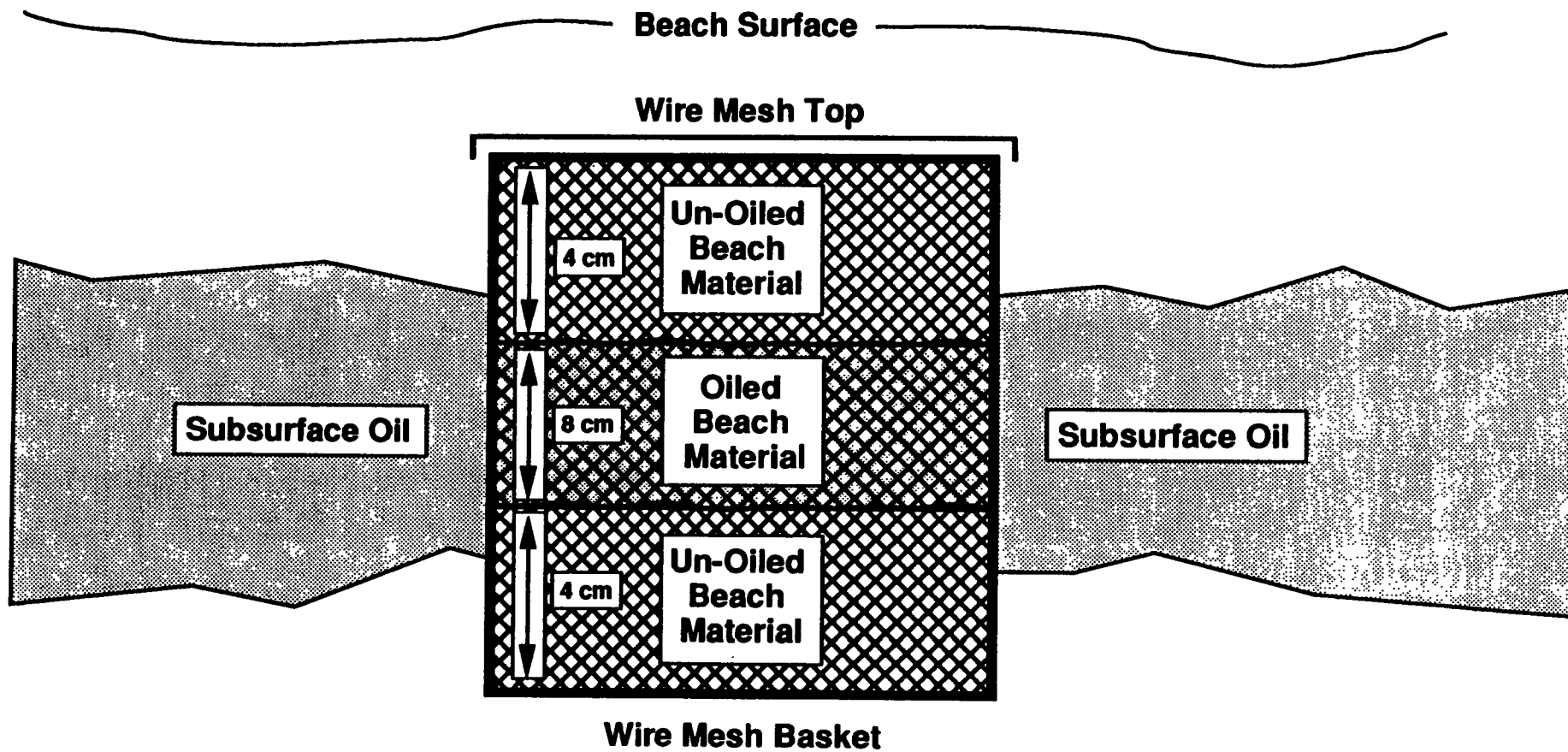
Figure 3.6.   Sampling Basket.

beach material (Disk Island) and the other with three layers of material: a 4 cm layer of oiled homogenate material sandwiched between two 8 cm layers of homogenized clean beach material (Elrington Island). The baskets were then implanted into fixed plots on a beach. The specific methods used to prepare sampling baskets in the field are described below:

- Oiled beach material was sieved through a 12.5 mm sieve, and the material that passed through the sieve was retained. The retained material was thoroughly mixed using hoes in a large plywood box for at least 20 minutes.

- Enough sample was randomly removed by using a hand trowel to fill each sampling I-chem jar. Six 1-kg samples of homogenized oiled beach material were collected from the homogenization box. Five samples of the oiled homogenate and three samples of the clean material from each plot were analyzed for oil chemistry; one of the oiled samples from each homogenate was analyzed in triplicate. The material was removed from a different area within the box each time. Samples were placed in I-chem jars, tightly capped, and stored in a cooler with frozen gel pacs. If microbiological analyses were not performed on the samples, dry ice was substituted for gel pacs to freeze the samples for oil chemistry analysis. Samples were returned as quickly as possible to Valdez for subsampling (if necessary) and analysis.

- If the mesh size of sampling basket exceeded the minimum size of the material, the basket was lined with fiberglass screening. The basket was then filled with the homogenized material and a lid was wired onto each basket.

- At Disk Island baskets were implanted in the beach plots with the top of the basket flush with the beach surface. At Elrington Island baskets were buried approximately 15 to 20 cm below the beach surface.

- For the subsurface oil layer baskets, the procedure outlined above was repeated, but clean beach material was substituted for oiled material. At least 3 subsamples of the homogenized clean beach material were collected. These baskets were filled as follows: a layer of clean material; a layer of oiled material; and a layer of clean material. The oil layer was about 8 cm thick and the top and bottom layers about 5 cm. Baskets designed for monitoring dissolved oxygen and nutrient uptake consisted of a well of unslotted PVC pipe placed approximately 2 cm into the bottom clean layer, and another unslotted PVC well placed at the interface of the oil layer and top clean layer. The wells were approximately 2 cm from the sides of the basket.

Disk Island

A diagram of the Disk Island beach used for this experiment is shown in Figure 3.7. Six 3 m x 3 m plots were marked with rebar and nylon rope (Figure 3.8). Four wells, each cased with 51 mm diameter slotted stainless steel, were installed in the center of the four treatment plots. Each well was 0.8 m from the center of the plot. The two outer untreated control plots each contained two wells.
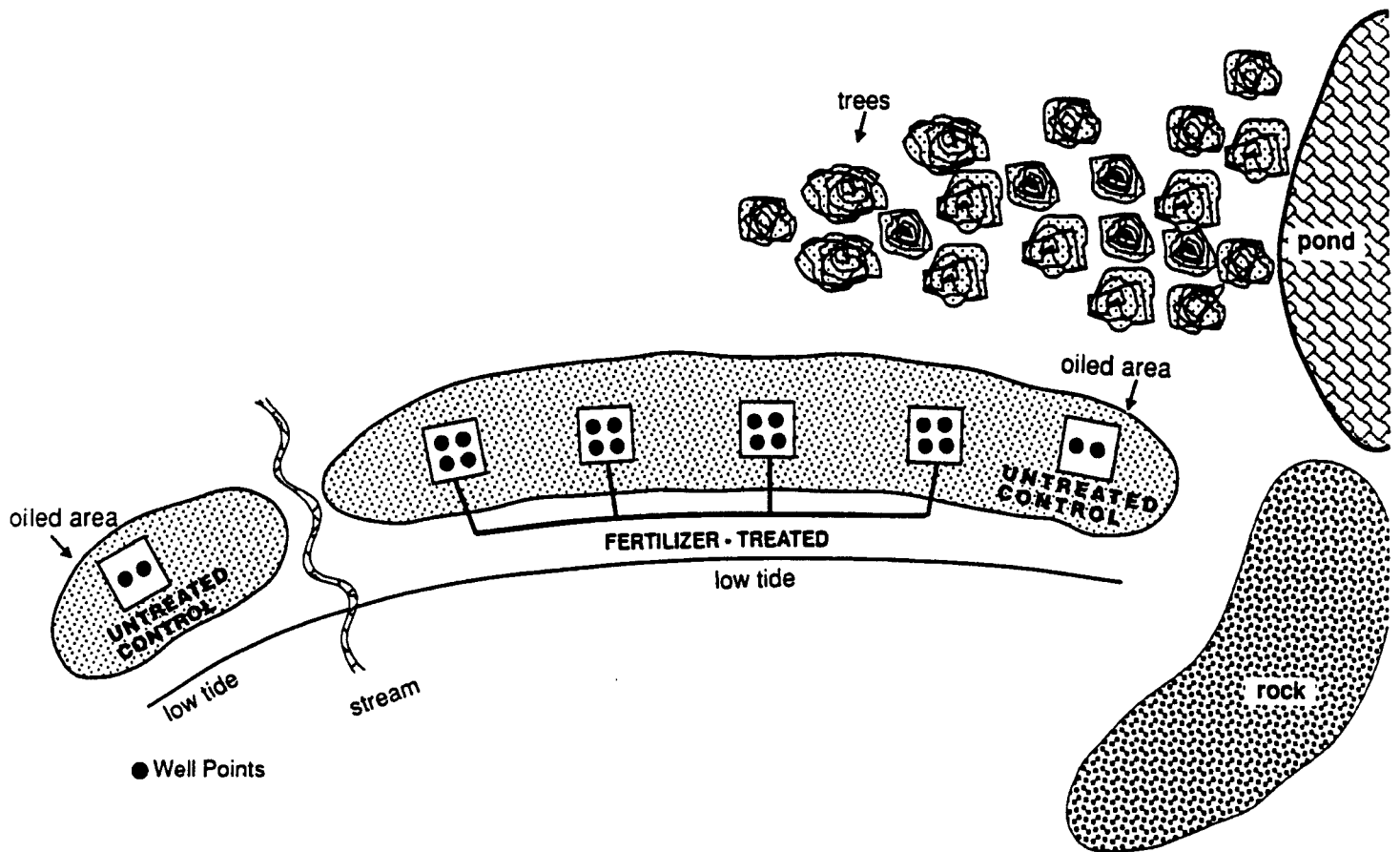
Figure 3.7.   Disk Island Schematic of the Beach for the Fertilizer Specific Activity Experiment.
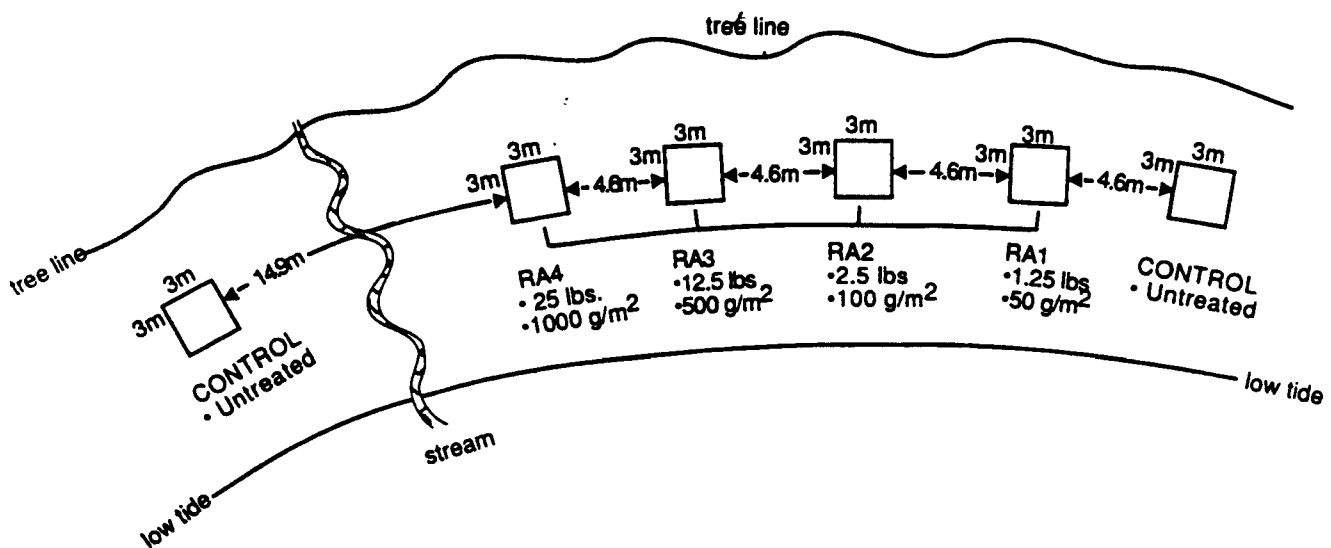
Figure 3.8.   Disk Island Fertilizer Specific Activity Plot Map and Rate of CUSTOMBLEN Granule Application.

Small, square sampling baskets containing homogenized oiled beach material were clustered around the wells on each of the plots. Material excavated for basket placement flush with the beach surface was removed from the test plot area. The four treatment plots received CUSTOMBLEN fertilizer at different application rates. Nutrient concentrations were monitored in the wells.

For the scaling experiment conducted in association with the Disk Island study, three plots on an uncontaminated cobble beach were marked with rebar and nylon: 3 m x 3 m, 6 m x 6 m, and 9 m x 9 m (Figure 3.9). Four wells, each cased with 1m lengths of 44 mm diameter slotted stainless steel, were installed in each plot, 0.6 m from the center of the plot so only the top unslotted 102 mm section remained above ground. The wells were capped to prevent tidal water from entering. Wells were numbered 1 though 4, beginning with the one farthest from the water line at low tide and proceeding clockwise. Each plot was treated with water-soluble fertilizer granules (CUSTOMBLEN). Nutrient concentrations were monitored in each well through time.

Elrington Island

Instead of marking plots, three areas of Elrington Island beach were delineated: the Bath beach, Sprinkler beach, and untreated Control beach (Figure 3.10). Beach experiment dimensions and rate of nutrient solution application are given in Figure 3.11. The Bath beach received one application of nutrient solution, the Sprinkler beach received multiple applications of nutrient solution, and the untreated Control beach received no treatment. A rock outcrop separated the Bath and Sprinkler areas. The Sprinkler and untreated Control beaches were separated by a 12 m buffer zone. Ten sampling baskets identical to those described for Disk Island above were arranged in a row on each beach. Larger cylindrical stainless steel baskets were used on the Sprinkler and untreated Control beaches; smaller square baskets were used on the Bath beach. Separate homogenates were prepared for each beach due to logistical constraints.

Nine of the baskets were used for microbiology and oil chemistry analyses. The tenth basket on each plot contained two wells cased with 51 mm diameter unslotted polyvinylchloride (PVC) pipe. These wells were used to monitor nutrient concentrations and dissolved oxygen at the upper and lower interface of the subsurface oil layer (Figure 3.12). Four wells were installed in both the Sprinkler and untreated Control beaches. The wells were located 0.6 m on either side of the baskets; wells on the same side were approximately 2 m apart.
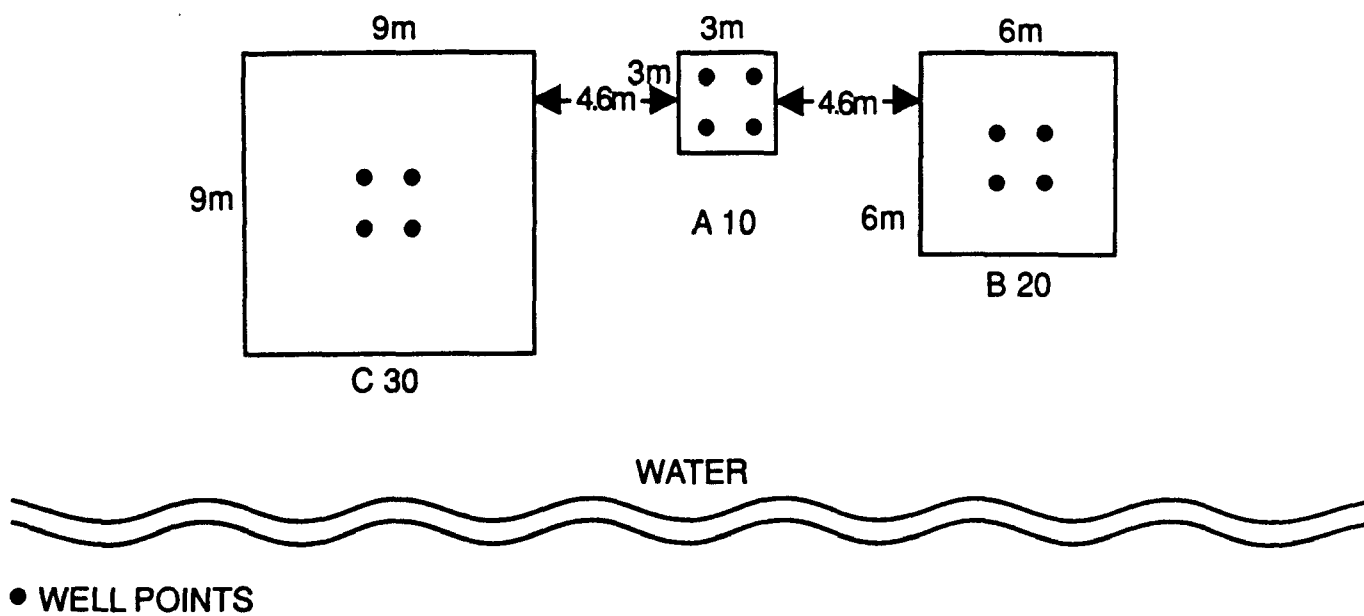
Figure 3.9. Scaling Experiment Plots on an Uncontaminated Cobble Beach.

Stream

Oiled Berm

Sprinkler Head

Oiled Area

Low Tide

BATH

Oiled Area

Oiled Area

Low Tide

SPRINKLER

Low Tide

CONTROL

Area Covered By
Feterlizer Solution
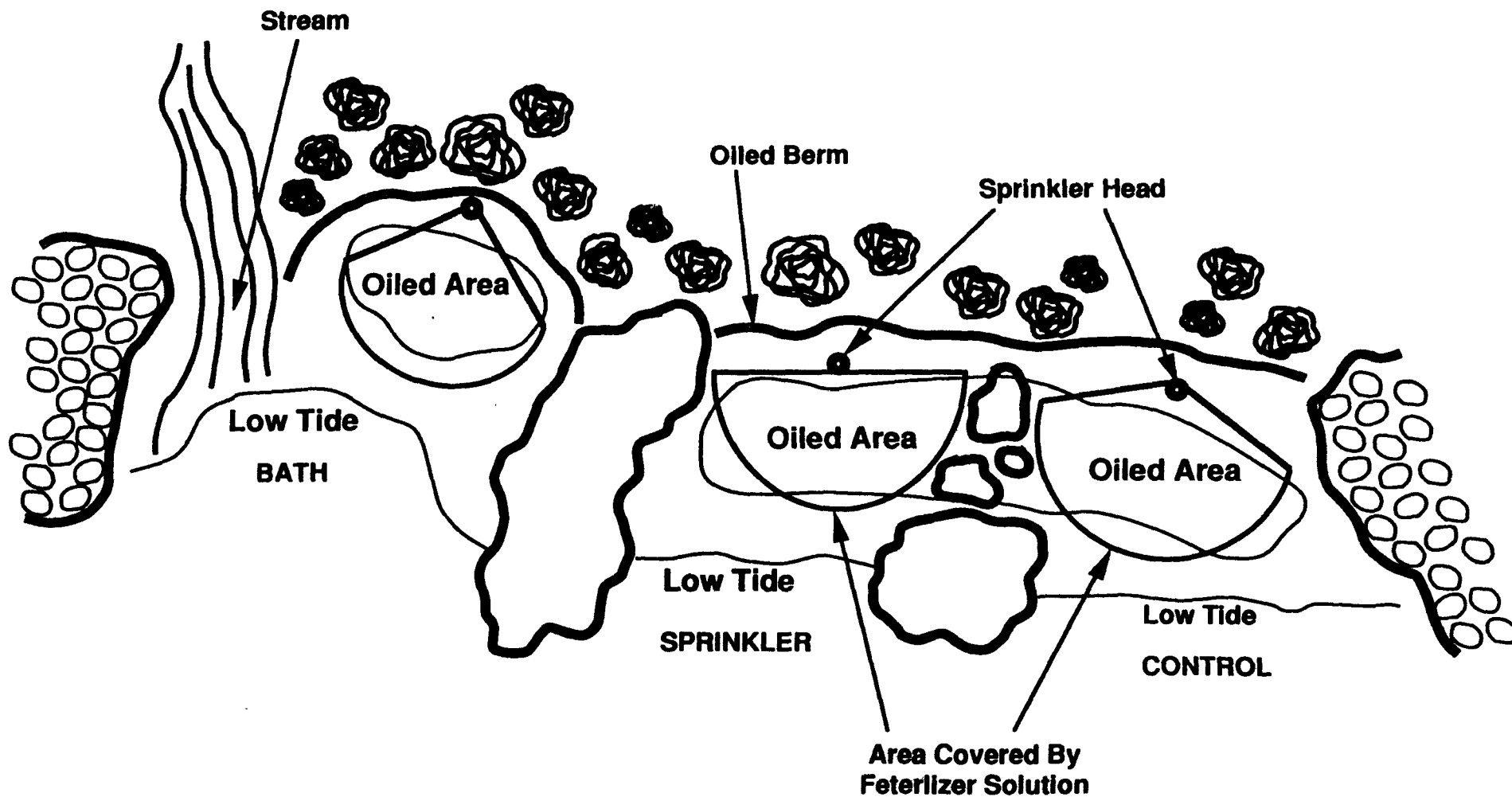
Figure 3.10.   Elrington Island Beach Diagram.

BERM **BERM** **BERM**

rock

low tide

low tide

low tide

Sprinkler Head
X

21.4 m

21.4 m

X

5.8 m

13.4 m

X

13.7 m

8 m

57

BATH

- 0.75 inches/hr
- 13.6 g/m² N
- 2.7 g/m² P
- 4 hour spray irrigation
- one application

SPRINKLER

- 0.4 inches/hr
- 6.88 g/m² N
- 1.37 g/m² P
- 4 hour spray irrigation
- six applications

UNTREATED CONTROL

- 0.4 inches/hr
- seawater only
- 4 hour spray irrigation
- six applications
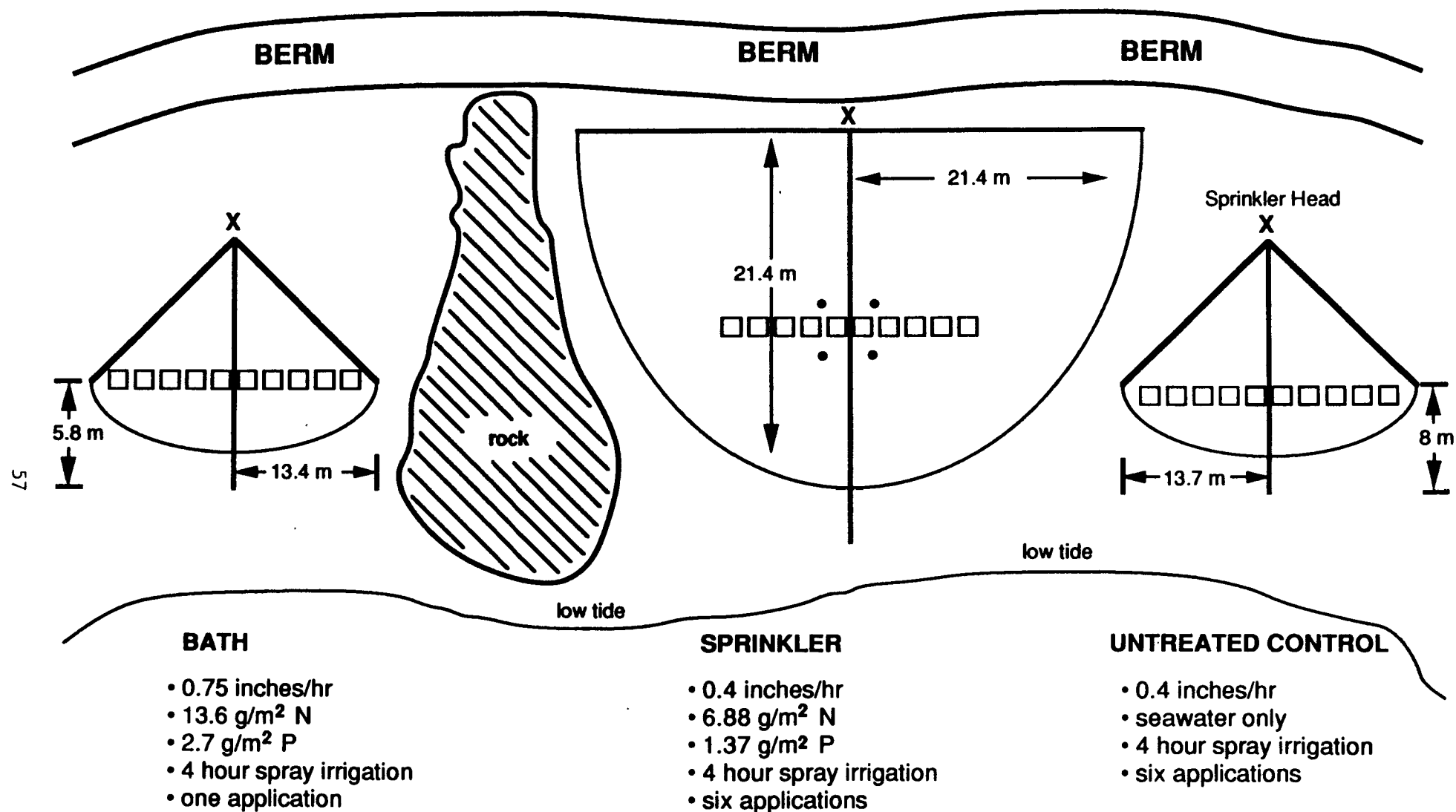
Figure 3.11. Elrington Island Nutrient Solution Experiment Beach Areas and Rate of Nutrient Solution Application.

DO Probe   DO Probe

5.08 cm PVC ⟶

Beach Surface

Wire Mesh Top

4cm

Un-Oiled
Beach
Material

8cm

Oiled
Beach
Material

4cm

Un-Oiled
Beach
Material
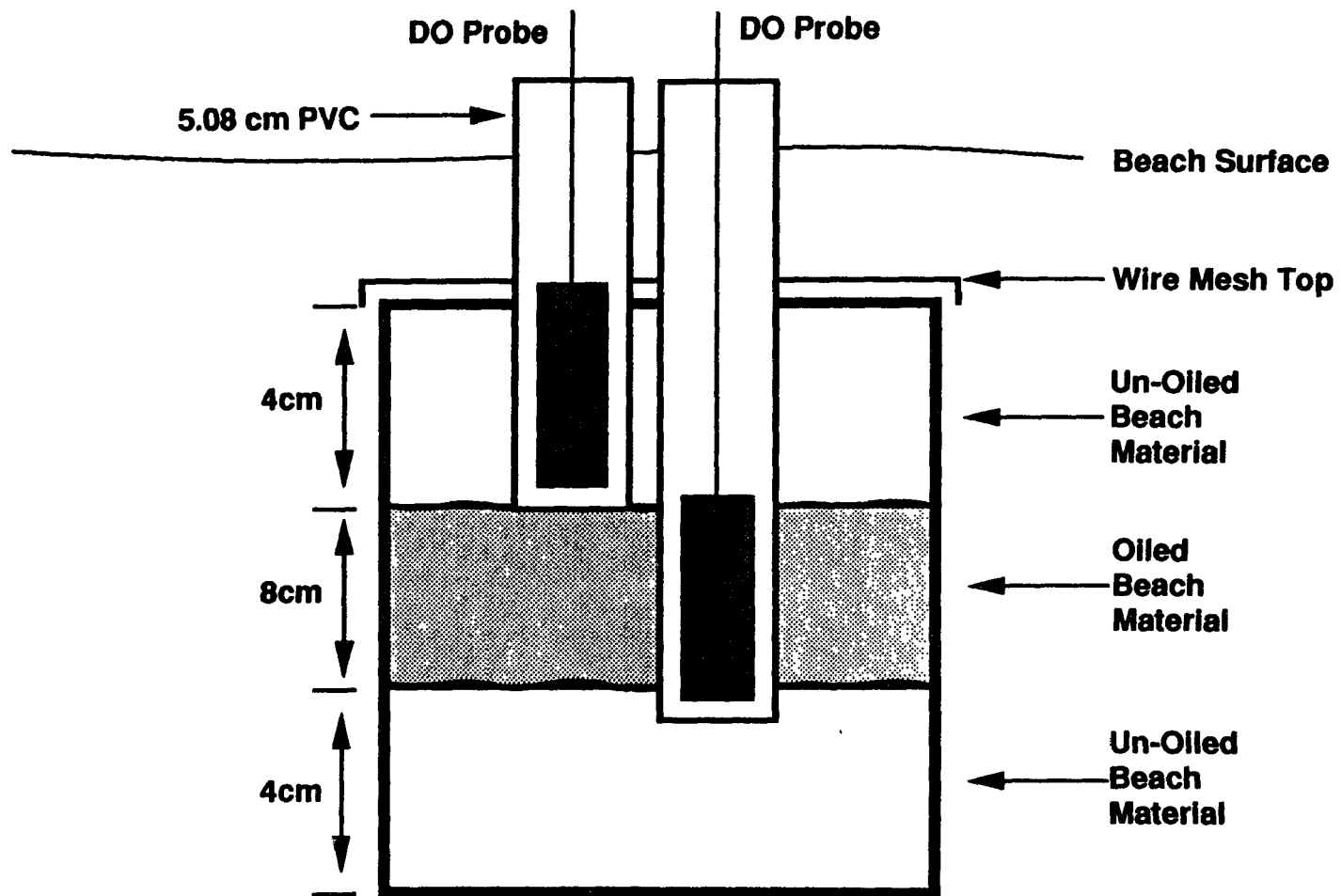
Figure 3.12.   Nutrient and Dissolved Oxygen Monitoring Basket.

Basket Removal and Sampling

Baskets were removed at set intervals from Disk Island and Elrington Island and subsampled for microbiology and oil chemistry analyses. Prior to application of the fertilizer, one basket was removed from each plot or beach on Disk Island to provide zero-time data for oil chemistry and microorganism activity. A timeline depicting basket collection from the test areas at Disk Island and Elrington Island is given in Figure 3.13. The schedule for basket collection depended on the experimental design. Baskets were removed from the beach, wrapped in aluminum foil, and placed in a small plastic garbage bag. The wrapped baskets were placed in a cooler with frozen gel packs. Dry ice was not used unless the basket was only used for oil chemistry analysis. Otherwise the baskets were returned to the lab immediately after collection and fully processed within 12 hours of the time of collection. Clean material was used to fill the hole left by the basket removal.

Subsampling of the baskets was performed in the laboratory in Valdez. Baskets which contained only oiled beach material (no layers of unoiled beach material) were subsampled as follows:

The top 1 to 2 cm of material was scraped off with a metal spoon and discarded. The beach material below was sampled in the middle of the basket with the spoon, avoiding material at the basket edges. If fertilizer granules were visible in the material, the first subsample was taken for oil chemistry to avoid fertilizer grains in samples for microbiological analysis. Oil chemistry samples were frozen in a glass jar at -20 °C. Samples for microbiology were contained in sealable bags or glass jars and were stored at 4°C. Any unusual observations (odor, visible color changes) were noted.

Baskets containing a subsurface oiled layer were sampled by first inserting a plastic ruler on one side of the basket to provide accurate depth measurements for removal of each layer. The top 25 mm of material was removed with a metal spoon, transferred to a sheet of aluminum foil, thoroughly homogenized with a spoon, and scooped into the sample containers. The subsampling procedure was repeated for each layer. Layers were defined as follows: a surface layer consisting of the top one inch of clean material; an upper oil interface located 13 mm on either side of interface; an oil layer located 25 mm from the middle of the layer; a lower oil interface located 13 mm on either side of interface; and a bottom layer located 25 mm from the bottom of the clean material layer. Any unusual observations (odor, visible color changes) were noted. Oil chemistry was conducted for all five basket layers from all baskets collected. Microbial respiration was measured in biometers for the upper oil interface, oil layer, and lower oil interface for two baskets from each plot within the beaches. Microbial activity was also determined for all five basket layers from all baskets collected.

DISK ISLAND
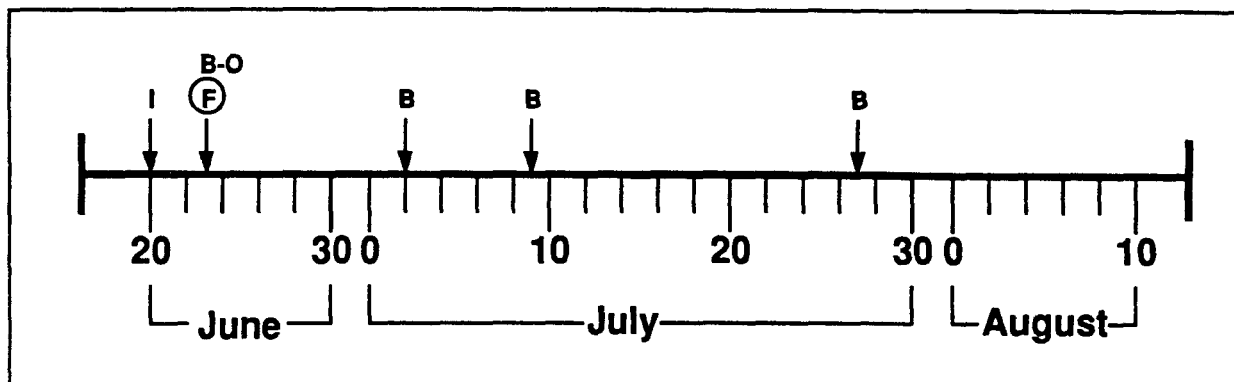
ELRINGTON ISLAND
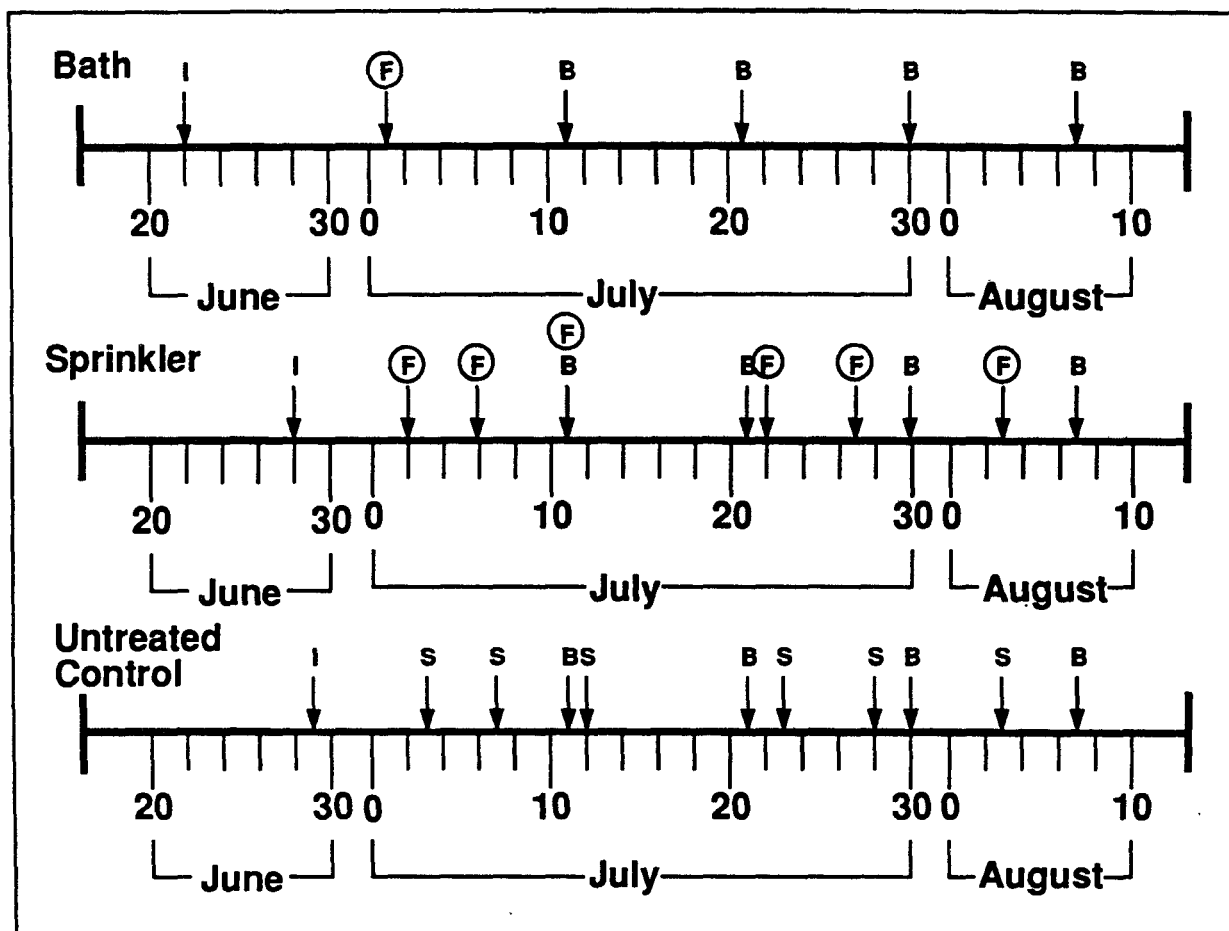
Legend

I = Date Baskets Were Installed in the Beach
F = Date of Fertilizer Application
B = Date of Basket Removal
B-O = Time 0 Basket Removal
S = Date of Seawater Application

Figure 3.13. Timeline Depicting Dates of Fertilizer Application and Basket Removal From Disk and Elrington Islands.

METHOD OF FERTILIZER APPLICATION

Slow-Release Fertilizers

Herring-seine net bags filled with slow-release fertilizer briquettes (WOODACE) were positioned on Otter and Seal beaches at Snug Harbor to provide complete exposure of the beach material to nutrients leached from the bags. Bags filled with WOODACE briquettes at Snug Harbor are shown in Figure 3.14. Each bag contained approximately 33 pounds of briquettes. Application of the briquette bags occurred on June 11, 1989. A timeline depicting fertilizer application to the test beach plots at Snug Harbor is given in Figure 3.15. The total quantity of briquettes applied to Otter Beach (35 m x 12 m test area) was 800 pounds, representing approximately 100 pounds nitrogen and 24 pounds phosphorus (as P_2O_5). The bags were tethered to 0.9 m sections of 2.9 cm diameter steel rods buried 15 cm below the surface of the beach. Figure 3.16a indicates the positioning of the 24 bags in the experimental area. Three rows of eight bags were placed at 2 m, 6 m, and 10 m from the top of the plot.

On June 20 and 21, 1989, the bags were repositioned according to the diagram in Figure 3.16b, as the bags located at the 2 m row were not consistently submerged by the high tide. In addition, preliminary data indicated that the nutrients were being channelled vertically down the beach. Four more bags were added to the previous 24 bags for a total of 28 bags, resulting in 920 pounds of fertilizer at Otter beach, or 130 pounds N and 30 pounds P (Table 3.4).

The same arrangement and repositioning was used for the briquette bags on Seal beach. This beach was smaller (28 m wide versus 35 m), so the weight of briquettes applied per bag was 26 pounds (versus 33 pounds) for a total of 620 pounds. This figure increased to 730 pounds when the four new bags were added, resulting in 103 pounds N and 22 pounds P (Table 3.4).

Slow-release fertilizer granules (CUSTOMBLEN) were applied to Tern beach in Passage Cove on July 25, 1989. Figure 3.17 shows a picture of the granules adhered to cobble at Passage Cove. A timeline depicting fertilizer application to the test beach plots at Passage Cove is given in Figure 3.15. The granules were applied using a commercial broadcast fertilizer spreader, at a rate of approximately 0.0033 lbs/ft^2. The total application of nitrogen and phosphorus was approximately 400 lbs and 40 lbs, respectively. The granules adhered to the oil on the rock surfaces and were therefore not easily displaced from the beach or redistributed by the tidal action. CUSTOMBLEN granules were also

TABLE 3.4. FERTILIZER TREATMENTS AT SNUG HARBOR, PASSAGE COVE, DISK ISLAND, AND ELRINGTON ISLAND

Beach or Plot within the beach	Treatment
Snug Harbor	
Otter Beach	130 lbs N, 30 lbs P per plot (WOODACE briquettes) Two applications[a] of INIPOL - 10 gallons (10.9 g/m^2 N and 4.4 g/m^2 P) and 10.5 gallons (11.5 g/m^2 N and 4.2 g/m^2 P)
Seal Beach	103 lbs N, 22 lbs P per plot (WOODACE briquettes) Two applications[a] of INIPOL - 13 gallons (10.7 g/m^2 N and 4.1 g/m^2 P) and 14 gallons (11.5 g/m^2 N and 4.4 g/m^2 P)
Eagle Beach	None- Untreated control
Passage Cove	
Tern Beach[b]	400 lbs N, 40 lbs P entire beach area (CUSTOMBLEN granules) plus INIPOL - 57 gallons (21.5 g/m^2 N and 8.2 g/m^2 P) Approximately 1,100 gal applied daily with
Kittiwake Beach	7 mg/L N and 3 mg/L P (Nutrient solution)
Raven Beach	None- Untreated control
Disk Island (Fertilizer Specific Activity)	
Control 2	None- Untreated control
RA4	1000 g/m^2 (CUSTOMBLEN granules)
RA3	500 g/m^2 (CUSTOMBLEN granules)
RA2	100 g/m^2 (CUSTOMBLEN granules)
RA1	50 g/m^2 (CUSTOMBLEN granules)
Control 1	None- Untreated control
Elrington Island	
Control Beach	Six applications of seawater only
Sprinkler Beach	Six applications of 6.88 g/m^2 N, 1.37 g/m^2 P (nutrient solution)
Bath Beach	One application of 13.6 g/m^2 N, 2.7 g/m^2 P (nutrient solution)

[a]INIPOL was reapplied because of the conditions under which it was originally applied (stormy and rainy).
[b]INIPOL values are best estimates.

Figure 3.14.   Bags Filled with WOODACE Briquettes at Snug Harbor.

SNUG HARBOR

Otter Beach

C° (Sept. 6)

Seal Beach

C° (Sept. 4)

PASSAGE COVE

Tern Beach

C° (Sept. 5)

Kittiwake Beach

C° (Sept. 5)

Raven Beach

C° (Sept. 5)
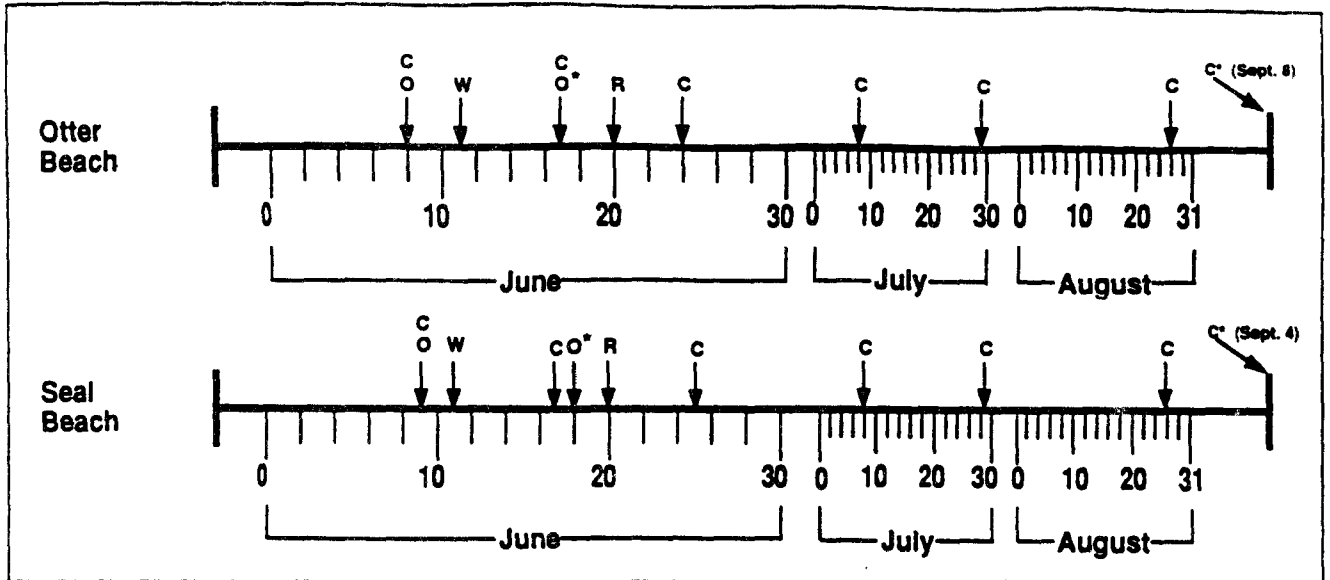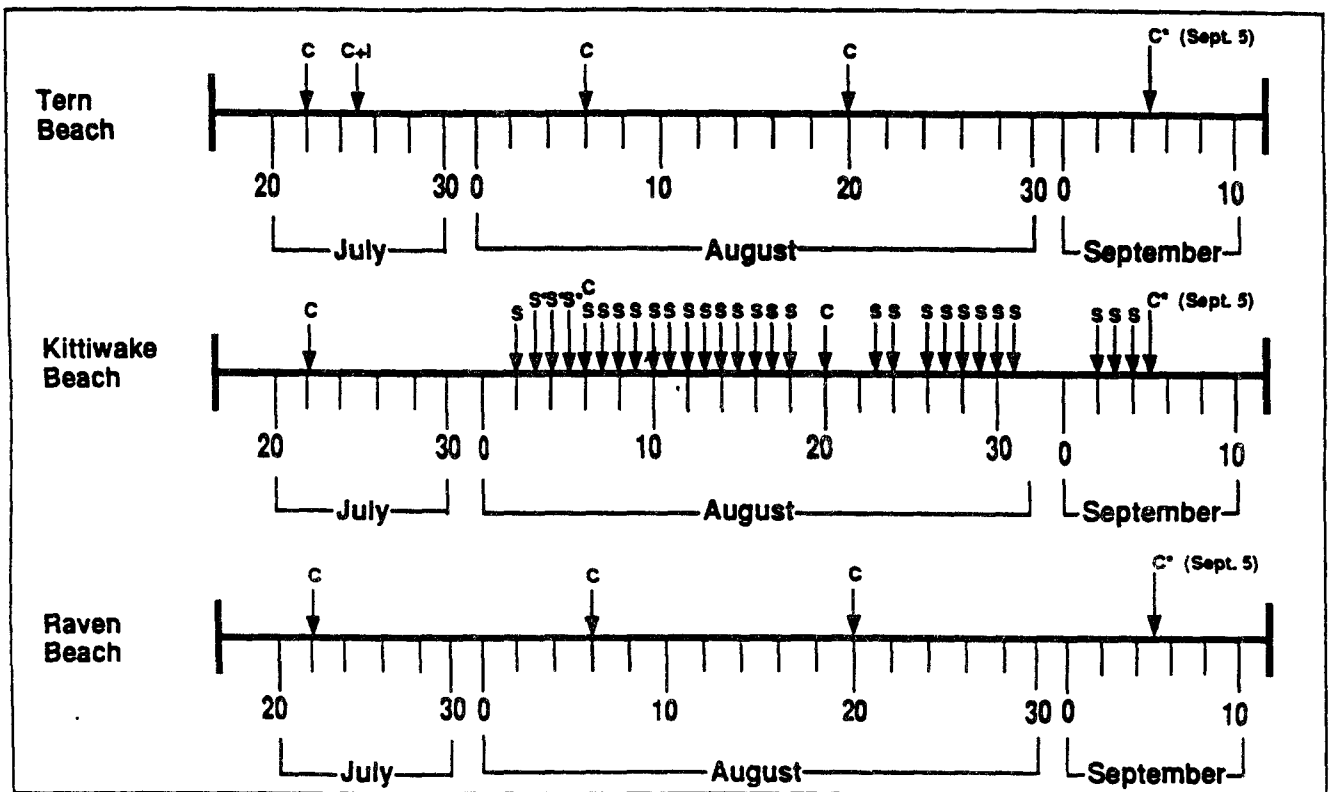
Legend

O = Oleophilic Fertilizer (INIPOL) applied
O° = INIPOL reapplied
W = Slow-release, WOODACE Briquettes applied
R = Nutrient bags repositioned and 4 nutrient bags added
C = Sampling date
C° = Final sampling date
C+I = CUSTOMBLEN granules + INIPOL applied
S = Fertilizer applied using sprinkler system
S° = 2 applications of fertilizer via sprinkler system

Figure 3.15. Timelines Depicting Dates of Fertilizer Application for Snug Harbor and Passage Cove.

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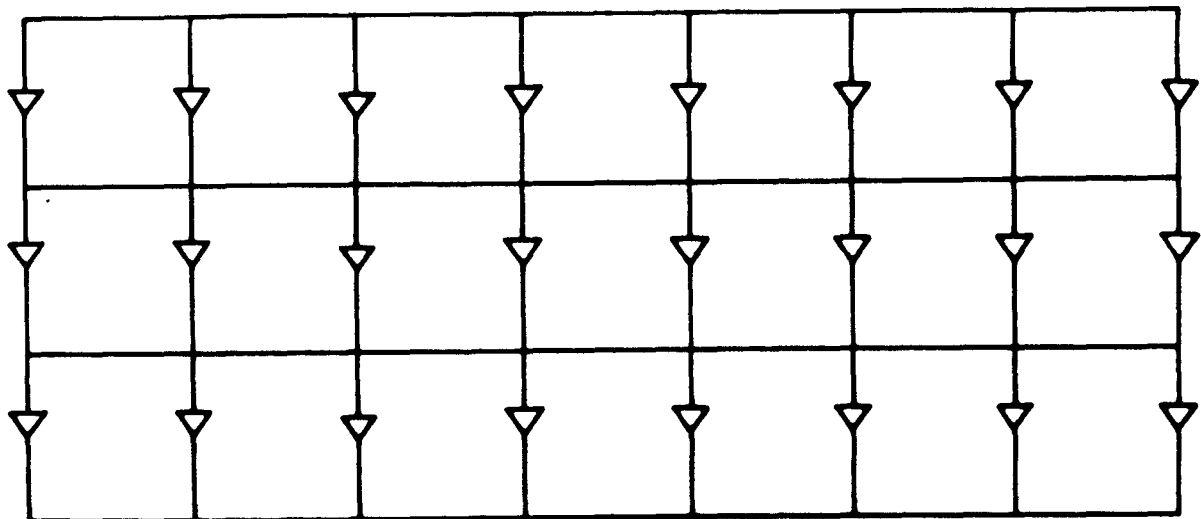
Figure 3.16A. Placement of the Bags of Fertilizer Briquettes on Otter and Seal Beaches (See Figure 3.2 for Beach Locations).
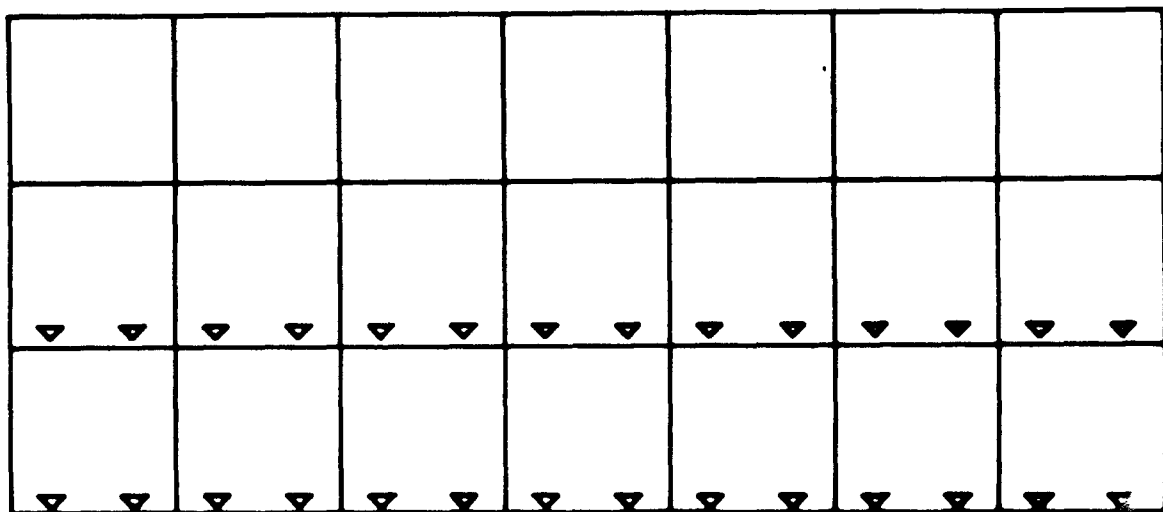
Figure 3.16B. Repositioning of the Bags of Fertilizer Briquettes on Otter and Seal Beaches (See Figure 3.2 for Beach Locations).
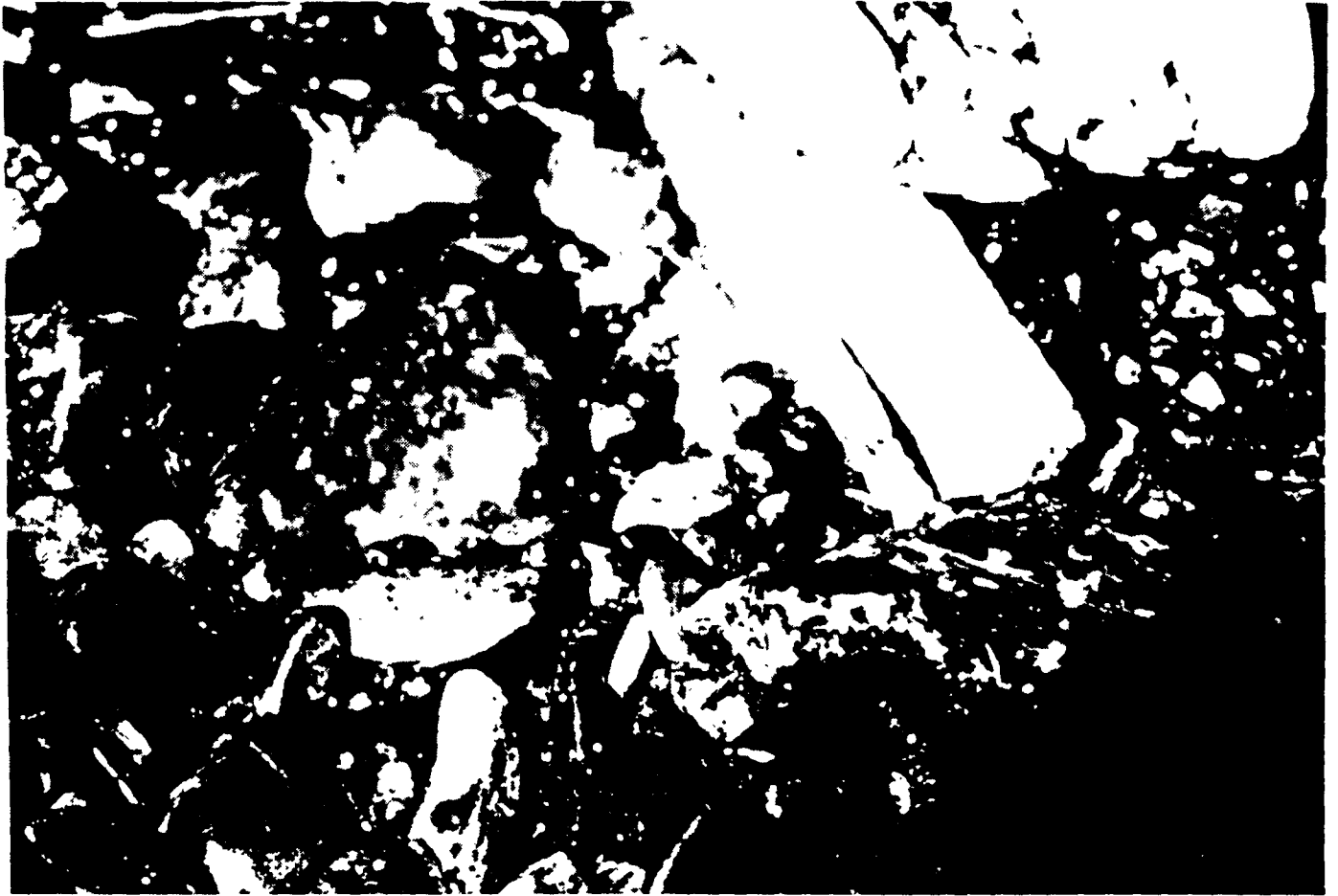
Figure 3.17. CUSTOMBLEN Granules Adhered to Cobble at Passage Cove.

applied to plots on Disk Island on July 1,1990, July 5, 1990, July 9, 1990, and July 13, 1990. A timeline depicting fertilizer application to these test beaches is given in Figure 3.10.

Oleophilic Fertilizer

The oleophilic fertilizer was applied using a backpack sprayer with a capacity of four gallons (Figure 3.18). The fertilizer was applied to both beaches as the tide was going out in the evening on the first application, and was initiated at the top of the beach an hour after the tide was past the lowest zone in the plot. A second application to both beaches occurred in the morning. The fertilizer was initially warmed, to ensure uniform application and prevent clogging of the spray nozzle.

Oleophilic fertilizer (INIPOL) was first applied to Otter beach in Snug Harbor on June 8, 1989 and to Seal beach in Snug Harbor on June 9, 1989 (Figure 3.15). The beaches received 13 and 10 gallons, respectively. The applications represented approximately 5% of the estimated weight of the oil on the beach. INIPOL was applied in combination with slow-release CUSTOMBLEN fertilizer granules to Tern beach in Passage Cove on July 25, 1989 (Figure 3.15). The following computations were made to determine the application rate:

For a plot 20 m x 12 m there are 240 m^2 or 2,600 ft^2. Assuming an oil depth of 6 inches, there is a total volume of oiled beach material of 1300 ft^3. From an estimated void volume of 20%, and a specific gravity of rock equal to 160 lbs/ft^3, this total volume contains approximately 160,000 pounds of rock. Based on a manufacturer's recommended 5% loading rate of the INIPOL relative to an estimated oil weight of 1% (1600 lbs of oil), 83 pounds or 10 gallons of INIPOL should be applied.

A second application of 10.5 gallons of INIPOL was performed on June 17, 1989, to Otter beach based on recommendations from Elf Aquitaine representatives. The second application to Seal beach occurred on June 18, 1989, at a rate of 14 gallons of INIPOL.

Fertilizer Solution

Kittiwake beach in Passage Cove was used to evaluate the effectiveness of daily nitrogen and phosphorus application via spray irrigation. A timeline depicting fertilizer application to Kittiwake beach is given in Figure 3.15. The sprinkler system began operating on August 2, 1989, using sprinkler heads typical of lawn sprinklers. The fertilizer was dissolved in seawater, and the solution

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Figure 3.18.   Application of Oleophilic Fertilizer Using a Backpack Sprayer.

was pumped by a gasoline-driven well pump to four sprinkler heads set on rebar stakes placed at approximately the midpoint on each side of the plot. Each sprinkler swept a 180° arc across the beach repeatedly during application (Figure 3.19). Typical applications consisted of 0.4 inches of water per day. A total of 17 pounds of ammonium nitrate fertilizer and 7 pounds triple-superphosphate fertilizer were applied. Application rates were established to supply 7 mg/L of nitrogen and 3 mg/L of phosphorus to pore water in the saturated beach material to a depth of 2 m. The pump delivered approximately 55 psi while operating, and pumped approximately 1100 gallons per hour.

At Elrington Island, fertilizer solution was also applied by a sprinkler system (see Figure 3.10 for application schedule). The system drew liquid fertilizer from a tank, mixed it with seawater, and applied the mixture at a predetermined rate over a 4-hour period at low tide. The treatment was reapplied approximately every 4 days on the Sprinkler beach, but only once on the Bath beach. The application rate information is given in Table 3.4. The sprinkler heads were commercially available heads used in agricultural irrigation systems. The same system was used to sprinkle unamended seawater over the untreated Control beach.

Over the 2-year period in which research was conducted, more than 5 field and 20 laboratory tests were implemented at several different sites in Prince William Sound. Table 3.5 summarizes all field tests conducted, including the beaches subjected to the various fertilizer treatments, and laboratory analyses performed. Table 3.6 summarizes the laboratory tests conducted, including the experimental design and laboratory analyses performed. The detailed descriptions of field and laboratory test designs and methods are described in Section 4.
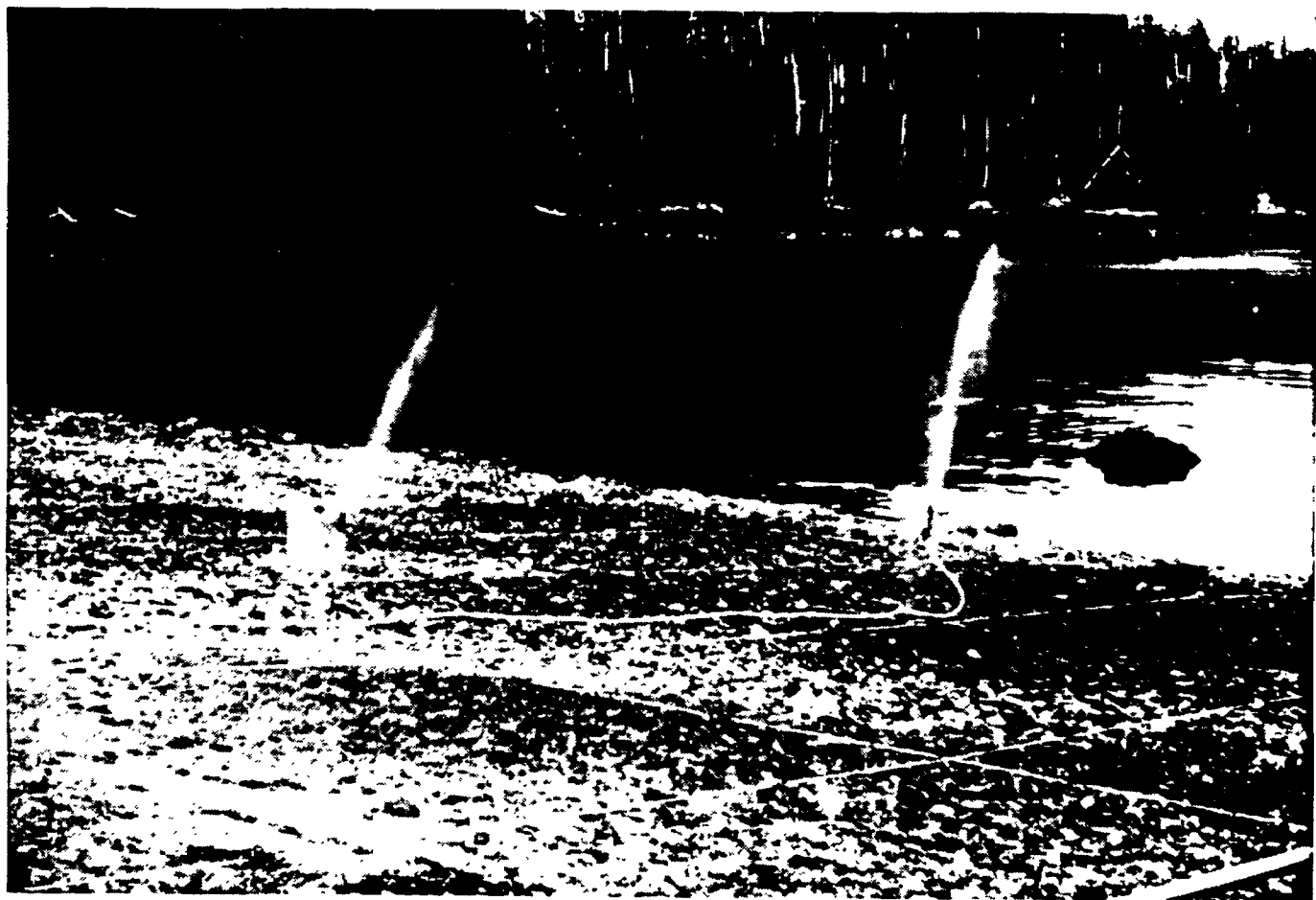
Figure 3.19. Sprinkler System in Operation at Passage Cove.

TABLE 3.5. SUMMARY OF FIELD TESTS

PART A - SUMMER OF 1989

Beach	Location	Beach/Type Oiling	Fertilizer Treatment	Analyses
Eagle	Snug Harbor	Sand, gravel/moderate	Untreated control- none	Oil chemistry (oil residue weight, composition); nutrients; microbiology (MPN); ecological (chlorophyll; phytoplankton primary prod; bact. abundance and produc; caged mussels)
Otter	Snug Harbor	Sand, gravel/moderate	INIPOL	Same as above
Otter	Snug Harbor	Sand, gravel/moderate	WOODACE briquettes	Same as above
Seal	Snug Harbor	Cobble over mixed sand and gravel/moderate	WOODACE briquettes	Same as above
Seal	Snug Harbor	Cobble over mixed sand and gravel/moderate	INIPOL	Same as above
Seal	Snug Harbor	Cobble over mixed sand and gravel/moderate	Untreated control- none	Same as above
Raven	Passage Cove	Cobble over mixed sand and gravel/heavily oiled- physically washed	Untreated control- none	Oil chemistry (oil residue weight, composition); nutrients; microbiology (MPN); ecological (chlorophyll; phytoplankton primary production; bact. abundance and produc; caged mussels; field toxicity tests)
Tern	Passage Cove	Cobble over mixed sand and gravel/heavily oiled- physically washed	INIPOL + CUSTOMBLEN granules	Same as above
Kittiwake Beach	Passage Cove	Cobble over mixed sand and gravel/heavily oiled- physically washed	Nutrient solution (sprinkler)	Same as above

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TABLE 3.5. (CONTINUED)

PART B - SUMMER OF 1990

Beach	Location	Beach/Type Oiling	Fertilizer Treatment	Analyses
Disk Island	Disk Island	Small cobble mixed into mixed sand and gravel	CUSTOMBLEN granules	Baskets: Oil chemistry (oil residue weight, composition); microbial activity (CO_2 produc., MPN); Wells: Nutrients; Ecological (intertidal food webs)
Bath	Elrington Island	Cobble overlying mixed sand and gravel/ Significant subsurface oil layer	One application of nutrient solution (sprinkler)	Baskets: Oil chemistry (oil residue weight, composition); microbial activity (O_2 consumption, CO_2 produc., MPN); dissolved O_2 uptake; Wells: Nutrients; Ecological (intertidal food webs)
Sprinkler Beach	Elrington Island	Cobble overlying mixed sand and gravel/ Significant subsurface oil layer	Multiple applications of nutrient solution (sprinkler)	Same as above
Untreated Control Beach	Elrington Island	Cobble overlying mixed sand and gravel/ Significant subsurface oil layer	Untreated control-none	Same as above
Seal Beach	Snug Harbor	Oil totally gone	CUSTOMBLEN granules	Wells: Nutrients

TABLE 3.6. SUMMARY OF SUPPORTING FIELD AND LABORATORY TESTS

PART A - SUMMER OF 1989

Experiment Type	Test Materials	Experimental Design	Analyses
Tank Microcosm Studies	Snug Harbor (mixed sand & gravel and cobble).	Effect of Oleophilic fert. (INIPOL) and soluble fert. in bags (not slow-release) on oil degradation.	Oil chem. (oil residue weight, composition); GC/MS.
Column Microcosm Studies	Mixed sand and gravel from Hell's Hole Beach (uncontaminated) and Raven Beach at Passage Cove.	Effect of INIPOL on oil movement and bacterial activity.	Numbers of oil-degrading microorganisms; Mineralization of ^{14}C labeled phenanthrene & hexadecane.
Jar Microcosm Studies	Snug Harbor.	Different nutrient mediums with and without INIPOL.	Numbers of oleic acid-degrading microorganisms; oil chem (oil composition).
Shake Flask Studies (Exxon)	Bushnell Haas Medium; Prince William Sound water; Alyeska ballast water; Artificially weathered Prudhoe Bay crude oil.	Effect of inocula in the presence of nutrients.	GC/FID.
	Artificially weathered Prudhoe Bay crude oil plus INIPOL and/or WOODACE Briquettes.	Effect of combined INIPOL and water-soluble nutrients.	
	Artificially weathered Prudhoe Bay crude oil plus INIPOL and Alyeska ballast water.	Effect of different concentrations of INIPOL; 3, 10, 20, and 50% of oil concentration.	

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TABLE 3.6. (CONTINUED)

PART A - SUMMER OF 1989

Experiment Type	Test Materials	Experimental Design	Analyses
Shake Flask Studies (Exxon) (cont'd.)	Artificially weathered Prudhoe Bay crude oil plus INIPOL or WOODACE Briquettes.	Effect of incubation temperatures (20 °C, 15 °C and 5 °C) in presence of INIPOL and soluble nutrients.	
	Prince William Sound oiled beach material plus INIPOL in poisoned and non-poisoned conditions.	Effect of INIPOL on oil degradation from oiled beach material.	
Respirometric Flask Studies	Artificially weathered Prudhoe Bay crude oil with uncontaminated beach material and Alyeska ballast water; or Snug Harbor seawater.	Relative comparison of the effects of INIPOL and soluble fertilizer and effects of inoculation.	Analytical respirometry; GC/FID and GC/MS.
Chemical Effect of Oleophilic Fertilizer (Exxon)	Oiled gravel.	INIPOL added to oiled rocks and oil release measured.	
Toxicity Tests	Laboratory-reared test organisms.	Toxicity of oleophilic fertilizer; Fish, invertebrates, or alga + a) INIPOL, seawater; b) INIPOL, artificially weathered Prudhoe Bay crude oil.	96-hour LC50.
Mutagenicity Tests	Snug Harbor mixed sand and gravel.	Mutagenic effect of oil beach material exposed to different fertilizers; INIPOL, briquettes.	Spiral *Salmonella* assay.

TABLE 3.6. (CONTINUED)

PART A - SUMMER OF 1989

Experiment Type	Test Materials	Experimental Design	Analyses
Food Chain Bioaccumulation of Carbon and Nitrogen	Biological samples from: Tatitalek Island, Snug Harbor, Passage Cove; Seston samples from: Snug Harbor, Passage Cove; Particulate matter from root feeders.	Movement of fertilizer nitrogen and oil carbon into food chain in sampling from fertilizer-treated and untreated beaches.	Biological: Stable isotope analyses of primary producers, consumers, and seston; Chemical: Stable isotope analyses of dissolved ammonium.

TABLE 3.6 (CONTINUED)

PART B - WINTER OF 1989/1990

Experiment Type	Test Materials	Experimental Design	Analyses
Biometer Flask Studies	Oiled beach material from Bay of Isles, artificial seawater.	Effect of nutrient concentration and timing of INIPOL application.	Cumulative CO_2 production; mineralization of ^{14}C labeled phenanthrene and oleic acid; oil chemistry (CH_2Cl_2/hexane extraction; gravimetric analysis; GC analysis).
Biometer Flask Studies Coupled to Micro-Oxymax Respirometer	*ibid.*	Effect of various concentrations of soluble nutrients day 1; N only; and P only.	Cumulative O_2 consump., cumulative CO_2 production; mineralization of ^{14}C phenanthrene; oil chemistry (CH_2Cl_2/hexane extraction; gravimetric analysis; GC analysis).
Biometer Flask Studies	*ibid.*	Effect of different agitation rates; 50, 75, 100 and 125 rpm.	Mineralization of ^{14}C phenanthrene; oil chemistry (CH_2Cl_2/hexane extraction of tidal-nate and rocks for gravimetric and GC analysis).
Biometer Flask Studies	*ibid.*	Different fertilizer application scenarios 1) INIPOL day 1 + soluble nutrients daily; 2) soluble nutrients daily; 3) soluble nutrients day 1 only; INIPOL day 1 only; control.	Cumulative CO_2 production; mineralization of ^{14}C phenanthrene & oleic acid.
Biometer Flask Studies	*ibid.*	Inoculation studies 1) Oil-enriched mixed culture + soluble nutrients; 2) Oil-enriched strain E12V + soluble nutrients; 3) Soluble nutrients only; 4) Prince William Sound water only.	Cumulative CO_2 production; mineralization of ^{14}C phenanthrene; CH_2Cl_2/hexane extraction of tidal-nate and rocks (gravimetric analysis; GC analysis).

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TABLE 3.6 (CONTINUED)

PART B - WINTER OF 1989/1990

Experiment Type	Test Materials	Experimental Design	Analyses
Toxicity Tests	Not Applicable.	1) INIPOL toxicity to wildlife (Acute LC50- quail);	1) Acute LC 50- to CUSTOMBLEN granules;
		2) INIPOL toxicity to fish;	2) 7 day LC50; survival & growth.
		3) Literature review: toxicity of INIPOL and constituents to mammalian and avian wildlife.	

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TABLE 3.6 (CONTINUED)

PART C - SUMMER OF 1990

Experiment Type	Test Materials	Experimental Design	Analyses
Biometer Flask Studies	Elrington Island, sampling baskets.	Mineralization of oil in samples from Bath Beach, Sprinkler Beach, Untreated Control Beach.	Microbial activity (CO_2 produc.) mineralization of ^{14}C labeled phenanthrene & hexadecane.
Column Microcosm studies	Elrington Island.	Control with oil; Control without oil pulse doses 20 ml nutrient solution; 2-hr doses 40 ml nutrient solution.	Nutrients; Cumulative CO_2 produc., TOC; oil chemistry (oil residue weight).
Microcosm Studies (Stable Isotopes)	Oiled gravel.	1) Fertilizer + seagrass detritus; 2) Fertilizer only; 3) Seagrass detritus only; 4) Control.	Nitrogen & carbon isotope ratios.
Stable Isotopes	Disk and Elrington Islands.	Fertilizer granules and fertilizer solution.	Biological: Stable isotope analyses of seston, bacteria; Chemical: Stable isotope analyses of ammonium, algae, consumers, predators.

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SECTION 4

CHEMICAL AND BIOLOGICAL ANALYTICAL PROCEDURES

Detailed information on the standard operating procedures are given in the Quality Assurance plans, prepared under the direction of the program Quality Assurance Officer, Dan Heggem (Papp et al., 1989; Chaloud et al., 1990). Only brief accounts of the analytical procedures have been included here.

NUTRIENT ANALYSIS

For the summer of 1989, water samples taken for nutrient analysis were filtered (Whatman glass fiber filter) and placed in 150 mL plastic screw capped bottles. The bottles were immediately frozen with a dry ice-antifreeze solution. Water samples taken offshore were collected with a clean bucket and subsamples were taken for nutrient analysis. Water samples from the beach were collected behind or in front of an ebbing or flooding tide, using a commercial root feeder. The root feeder was outfitted with rubber tubing and a peristaltic pump to allow interstitial pore water to be drawn into the feeder tube and sampled at the top of the feeder tube. The feeder was inserted approximately 20 cm into the mixed sand and gravel. Pore water was flushed through the feeder for one minute prior to sampling.

For the summer of 1990, interstitial water samples were collected from monitoring wells using a peristaltic pump. Samples were collected as the incoming tidal water filled each well and/or after the outgoing tide had exposed the wells. A brief lag was required following outgoing tide well exposure in order to allow excess tidal water to percolate through the beach material. The nutrient sampling schedule was experiment-specific and is summarized in Table 4.1.

To collect water samples, tubing was inserted below the water line in the well. The pump was turned on and allowed to run for a full, slow count of 10 (10 to 15 seconds). Pumped water was discarded away from the test area. After this rinse, the sample container was rinsed with approximately 25 to 50 mL of pumped water, capped, and vigorously shaken, ensuring that all interior surfaces were contacted. The rinse was discarded. Sample bottles were filled to within 1.3 cm of the top, tightly capped, and placed in Ziploc bag. The bottles were immediately stored in cooler with frozen gel packs. Water collection was repeated for each well.

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TABLE 4.1. NUTRIENT SAMPLING SCHEDULE FOR SNUG HARBOR, PASSAGE COVE, DISK ISLAND, AND ELRINGTON ISLAND

Snug Harbor (Root Feeder Interstitial Water Samples)

Ammonia - Samples taken on both the incoming and outgoing tide

1) Prior to fertilizer application (T=0);
2) One to two days post application (T=1);
3) Eight to ten days post application (T=2);
4) Thirty days post application (T=3); and
5) Six weeks post application (T=4)

Nitrate/Nitrite - Samples taken on both the incoming and outgoing tide

1) One to two days post application (T=1);
2) Eight to ten days post application (T=2); and
3) Thirty days post application (T=3)

Passage Cove

   Data lost.

Disk Island (Well Samples)

1) Pretreatment (zero-time data);
2) Incoming tide following treatment;
3) Next outgoing tide;
4) Every other outgoing/incoming tidel thereafter, through Day 4 (fourth day following fertilizer treatment); and
5) An incoming and an outgoing tide on days 8, 12, and 16.

Elrington Island (Well and Basket Samples)

1) Samples were taken from the wells on each of the 3 days between applications on the Sprinkler plot;
2) Samples were taken from the monitor baskets on the day preceding nutrient application.

Within a few hours after collection, each sample was filtered through a 0.7μ GFF filter to remove aquatic organisms and particulate matter into one 250-mL and one 125-mL prewashed polyethylene or Nalgene wide-mouth sample bottles. The filter apparatus is shown in Figure 4.1. After filtration, all samples for nutrient analysis were stored at -20°C. If samples were not filtered within 12 hours after collection, they were frozen in the field using dry ice.
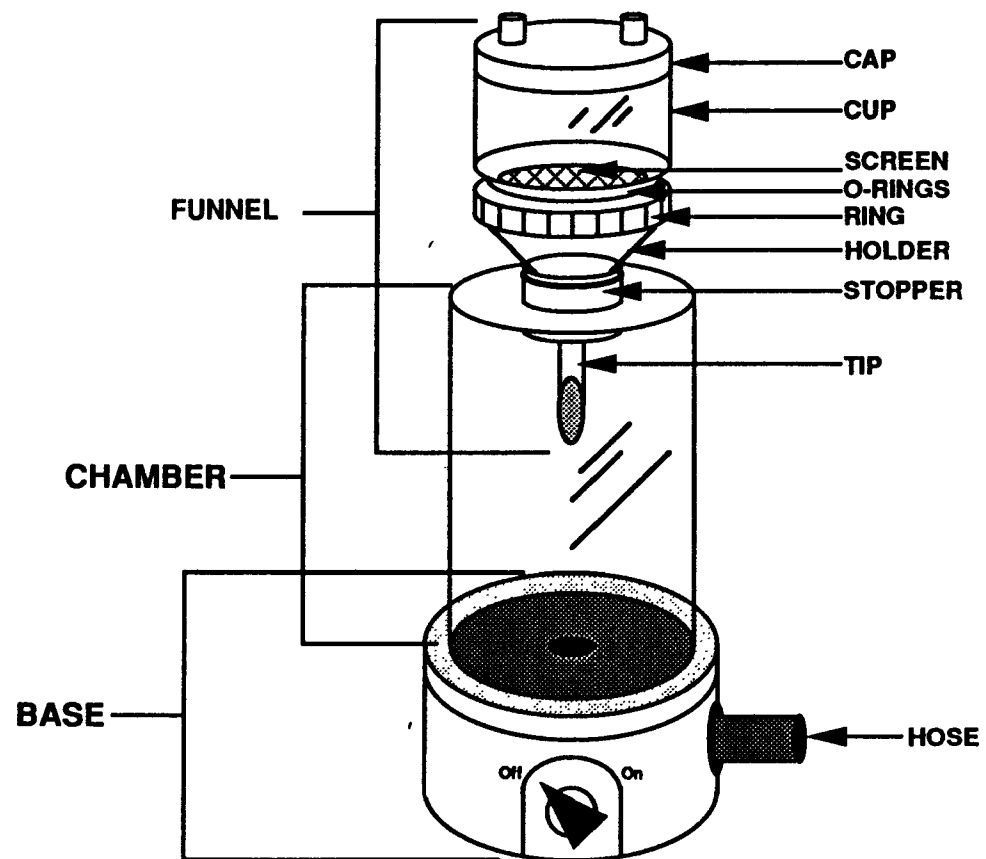
Figure 4.1. Nutrient Sample Filtration Apparatus.

Bulk water samples used in ecological monitoring and microbiology analyses for summers 1989 and 1990 were filtered using a 0.2 μ filter. Filtration was performed immediately after collection using a standard filtration set-up with either a peristaltic or vacuum pump.

Phosphate, ammonia, nitrate, and nitrite were measured in all field samples; only phosphate and ammonia were measured in laboratory-generated samples. The standard methods outlined below were designed to quantitate the total amount of phosphorus in the form of phosphate and the total amount of nitrogen in the form of nitrate, nitrite, and ammonia.

Nitrite and Nitrate

Nitrite was determined by the Griess reaction in which sulfanilamide and N-(1-Naphthyl) ethylenediamine dihydrochloride (NNED) is reacted with nitrite in an aqueous acidic solution to form an intense pink diazo dye with an absorption maximum of 540 to 543 nm. This method was also used for nitrate, following initial reduction to nitrite by passing through a column containing copperized cadmium fillings (Parsons et al., 1984). Detection limits for nitrate and nitrite were expected to be 0.05 μ and 0.01 μ, respectively.

Ammonia

Ammonia was determined by the Colorimetric Phenate method or Phenol-Hypochlorite method, in which hypochlorite and phenol react with ammonium in an aqueous alkaline solution to form indophenol blue, an intensely blue chromophore with an absorption maximum at approximately 637 to 640 nm (Parsons et al., 1984). The detection limit for ammonia was expected to be approximately 0.1 μ.

Phosphate

Phosphate (i.e., orthophosphate) was determined as phosphomolybdic acid, which has an absorption maximum at 880 to 885 nm in its reduced form in the presence of antimony (Parsons et al., 1984). The detection limit for phosphate was expected to be 0.03 μ.

OIL CHEMISTRY

Beach samples for chemical analysis consisted of either mixed sand and gravel contained in 400 mL I-Chem jars or cobblestones wrapped in aluminum foil and frozen prior to analysis. The mixed sand and gravel was thawed immediately prior to the initiation of oil analysis, and the contents were mixed thoroughly.

The following procedures were intended to assess the total amount of oil degradation and the change in hydrocarbon composition as a result of biodegradation.

Oil Residue Weight

Figure 4.2 shows the detailed steps of this extraction scheme.

A 100 g subsample of mixed sand and gravel was removed from the I-chem jars and mixed thoroughly with methanol in an Erlenmeyer flask. The slurry was shaken for five minutes, and the methanol decanted out of the Erlenmeyer flask. The samples were similarly reextracted two times with HPLC grade methylene chloride. The weight of extracted mixed sand and gravel was determined by drying. The organic fractions were combined and backextracted with 3% aqueous sodium chloride. The phases were separated and the aqueous portion was extracted with fresh methylene chloride. All methylene chloride extracts were combined.

Several boiling chips were added to the methylene chloride and the volume of solvent was reduced using a three-ball Snyder column attached to the round-bottom flask heated on a steam bath. Volume was reduced until the color was approximately the color of dilute weathered oil (ca 15 mg/2 mL methylene chloride). The final volume of the extract was measured and an aliquot was transferred to a GC autosampler vial.

All cobblestones were extracted using the same procedure (methanol, followed by methylene chloride), except that shaking was replaced by gentle swirling to remove oil from the rock surfaces.

A measured aliquot of methylene chloride extract was allowed to dry for residue oil weight analysis. For selected samples, a 2-mL aliquot of the methylene chloride extract was extracted with hexane. The hexane extract was then brought to dryness and the residue weighed. These analyses

Sand / Gravel (ca. 100 g)
|
Extract With 75 mL MeOH/5 min ———————
|
Extract With 75 mL DCM/5 min ——————
|
Extract With 75 mL DCM/5 min —————
|
Sediment Dried and Weighed

DCM / MeOH
|
Extract With 75 mL 3% NaCl
| |
DCM   NaCl / MeOH
| |
| Extract With 25 mL DCM
| | |
| DCM   Discard
| NaCl / MeOH
|_____|

Condense DCM to ≥ 3.00 mL
(Measure Volume)
|
|———————► Remove Measured Volume (2.00 mL) for Residue Weight Analysis
|
N$_2$ Blowdown of Remaining DCM To Dryness
|
Add 10 mL Hexane, Vortex Mix, Centrifuge
|——————————————————► Hexane Supernate
Add 10 mL Hexane, Vortex Mix, Centrifuge |
|——————————————————► Hexane Supernate
Add 10 mL Hexane, Vortex Mix, Centrifuge |
|——————————————————► Hexane Supernate
| |
Discard   Condense Combined Hexane Supernates (≥3.00 mL)
Pellet   (Measure Volume)
                                |
                                |———►Remove
                                | Measured Volume (2.00 mL)
                                | For Hexane Extractable Weight
                                | Analysis
                         Analyze By FID-GC

Figure 4.2. Oil Chemistry Sample Extraction.

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provided hexane extractable residue weight, and the residual hexane-unextractable component. The hexane unextractable residue weight was assumed to be asphaltenes, which are not degradable by bacteria.

Oil Composition

Hydrocarbon analysis of methylene chloride extracts or the hexane extractable fraction was determined by GC/FID. The conditions for the GC during the summer of 1989 were as follows:

Column: DB5, 30 m X 0.25 mm, film thickness 0.25 μ
Initial Temperature: 45°C, 5 minute hold
Temperature Rate: 3.5°C/minute
Final Temperature: 280°C, 60 minute analysis
Injector: Splitless, 1 minute valve closure
Injector Temperature: 285°C
Injection: 2.0 μL
Detector: FID, 350°C

The conditions for the GC during the summer of 1990 were similar, but had a 30 m X 0.32 mm column, a final temperature of 280 °C with a 20 minute hold, and a 1.0 μL injection.

Respirometric Studies

Methylene chloride was added to sample seawater in a volume ratio of 1:10 methylene chloride:seawater. The sample was extracted using EPA SW 846 Method 3510 (separatory funnel method): the seawater sample was transferred into a 250 mL separatory funnel; 1 mL of a 50 ppm HC surrogate standard and 1 mL of 1 ppm PAH surrogate standards were added. The sample bottle was rinsed with 30 mL methylene chloride and the extract added to the separatory funnel. The funnel was sealed and shaken for 1 to 2 minutes; the organic layer was allowed to separate from the water phase and the methylene chloride extract was collected. The extraction was repeated twice using 30 mL of methylene chloride, passed through an anhydrous sodium sulfate column, combined in an evaporation concentrator, and condensed to a final volume of 1 mL. The extract was then passed through a column of silica gel and again concentrated to 1 mL. Aliphatic hydrocarbons were analyzed using GC/FID under the following GC conditions:

```
Column:  DB-5, 0.75 mm ID X 30 m
Initial Temperature:  50°C, 5 minute hold
Temperature Rate:  7°C/minute
Final Temperature:  300°C, 75 minute or less analysis
Injector:  Splitless
Injector Temperature:  250°C
Injection:  2.0 µL
Detector:  FID, 350°C
```

Aromatic hydrocarbons were analyzed using CD/MS under the following GC conditions:

```
Column:  DB-5, 0.25 mm ID X 30 m
Initial Temperature:  50°C, 5 minute hold
Temperature Rate:  8°C/minute
Final Temperature:  300°C, 50 minute analysis
Injector:  Splitless, 0.8 minute valve closure
Injector Temperature:  270°C
Injection:  2.0 µL
Detector:  MSD, 350°C
Selected Ion Monitoring (SIM) mode with 100 msec dwell time
```

A slightly different analytical procedure was used for laboratory oil biodegradation studies conducted during the winter of 1989/1990. One hundred mLs of methylene chloride were added to biometer flasks at the end of an experiment, shaken (200 rpm) for 1 min, and the organic phase transferred to a clean flask. This extraction procedure was repeated two more times with 50 mL volumes of methylene chloride. Combined organic phases were passed through a layer of anhydrous sodium sulfate (ca. 25 g) to remove residual water and suspended solids. An aliquot of known volume was transferred to a clean (methylene chloride-rinsed) tared 25 mL test tube, and methylene chloride was removed under a stream of dry nitrogen at 25 to 30°C. Residual solvent was removed by placing tubes in a desiccator for 48 hours and the tubes were weighed to determine the amount of methylene chloride-extractable residue.

Methylene chloride-extractions were subsequently extracted 3 times with 10 mL volumes of hexane. Separation of hexane-soluble/methylene chloride-soluble materials (hexane insoluble fraction) was facilitated by centrifugation (5000 rpm, 10 min). Hexane-soluble fractions were transferred to clean, tared test tubes and hexane was removed under a stream of dry nitrogen at 30°C. Residual solvent was removed from both the hexane-soluble and hexane-insoluble fractions under desiccation. Hexane-soluble fractions were weighed, and changes in the chemical profile of this fraction were determined by gas chromatographic analysis.

"Tidal waters" from flask experiments were also extracted with methylene chloride as described above.

MICROBIOLOGY

Numbers of Oil-Degrading Microorganisms

The Most Probable Number (MPN) technique was used to determine the number of total heterotrophic and hydrocarbon-degrading microorganisms. Numbers of oil-degrading microorganisms were measured at Snug Harbor and Passage Cove (summer 1989) by an extinction to dilution procedure using oil as the carbon source.

The defined nutrient medium used in these tests contained (per liter of distilled water): NaCL, 24 g; $MgSO_4.7H_2O$, 1.0 g; KCl, 0.7 g; KH_2PO_4, 2.0 g; Na_2HPO_4, 3.0 g; and NH_4NO_3, 1.0 g. The pH of the medium was adjusted to 7.4 with 1.0 N NaOH following autoclaving. The medium was distributed in 4.5 mL portions to sterile dilution tubes. Initial dilutions were prepared by adding 5.0 g wet weight of sand and gravel subsample to the prepared dilution bottles containing 50 mL autoclaved defined.nutrient medium. Following vigorous mixing by hand for 15 seconds, a 0.5 mL sample of the initial dilution was used to prepare a dilution series from 10^2 to 10^{10}. Each tube was then amended with 20 μL of sterile weathered Prudhoe Bay crude oil collected from an oil-contaminated beach in Prince William Sound. Tubes were incubated at approximately 15°C for 21 days with 15 second shaking every three days. The tubes were scored independently by two individuals at 21 days of incubation. Tubes that showed visible microbial turbidity or changes in the physical form of the oil (oily droplets converted to stringy and flaky particulate material) were considered positive. Numbers of oleic acid-degrading bacteria were determined using standard plate counting procedures on defined nutrient agar medium supplemented with 1% oleic acid.

The standard "5-tube" MPN (APHA 1985), as modified for hydrocarbon-degrading microorganisms and field considerations, was employed during the summer of 1990. Hydrocarbon-degrading microorganisms were defined as those capable of emulsifying a Prudhoe Bay oil sheen layered on Bushnell-Haas marine mineral salts broth. Total heterotrophs were defined as those capable of growth (turbidity) in marine broth.

The MPN technique required inoculation of five 100 μL aliquots of each serially diluted sample into sterile 24-well microtiter plates containing approximately 1.75 mL of sterile broth. Following inoculation, a sheen of sterile Prudhoe Bay crude oil was applied to each well of the Bushnell-Haas plates. Each microtiter plate was incubated at 16 ± 2°C for three weeks following inoculation. Wells were scored as positive when oil emulsification was clearly indicated by disruption of the sheen.

Based on the results of a number of replicate inoculations (typically either three or five), the statistically significant MPN of microbes (selected for or by the medium) per unit volume was calculated. If the numbers fell below or above the dilution series selected, then the final numbers were reported as either less than or greater than the table value.

Mineralization of Radiolabeled Hydrocarbons

Evolution of $^{14}CO_2$ from phenanthrene-9-^{14}C, a polynuclear aromatic compound, hexadecane-1-^{14}C, a straight chain aliphatic, and naphthalene-1-^{14}C was used to measure the activity of indigenous petroleum-degrading microorganisms as influenced by the addition of oleophilic (INIPOL) and water-soluble fertilizers. Duplicate 5.0 g samples of beach material (1 to 5 mm diameter) obtained from oiled beaches with and without fertilizer treatments were added to 10 mL artificial salt-water medium (ASWM) in clean, sterile 100 mL Wheaton bottles. Each bottle was spiked with 0.1 μCi of radiolabeled substrate and crimp sealed with a Teflon-lined septum.

Following 0, 12, 24, and 48 hour incubation in the dark at ambient temperature (ca. 15°C), vessels were sacrificed and the amount of radiolabeled CO_2 was determined by acidifying the medium to pH<3.0 with HCl, flushing the headspace for 10 minutes with N_2 gas, and trapping CO_2 in 5.0 mL of 1 N NaOH. Subsamples (0.5 mL) of NaOH trapping solution were added to 10.0 mL Ready-safe liquid scintillation cocktail, and the amount of radioactivity present was determined by liquid scintillation. Trapping efficiency was determined by recovery of $^{14}Na_2CO_3$ from acidified medium. Quench was accounted for internally.

Hexadecane oxidation potential associated with sediment microorganisms was assayed only during the summer of 1990. To determine hexadecane oxidation potential, a 40 g (wet weight) portion of each of the sediment samples was mixed in a sterile 500-mL flask containing filtered and sterilized PWS seawater. After vigorous shaking by hand for one minute, 10 mL aliquots of the sediment slurry were pipetted into sterile 40 mL precleaned glass incubation vials fitted with Teflon-

lined septa. Each vial was injected with 5 or 50 μL of a 20 g/L solution of a radiolabeled hexadecane (in acetone). The resulting initial concentration of added hydrocarbon was 10 or 100 μg/vial (wet sediment). One mL of 4N HCl was injected into one vial of each series at time zero to determine the amount of radiolabel added. The remaining vials in each series were incubated without shaking for 1, 2, and 5 days with 1 or 10 ppm radiolabeled substrates. All incubations were conducted in the dark at 16 $\pm$ 2°C.

The extent of hydrocarbon transformation was measured by recovering the $^{14}CO_2$ produced from the ^{14}C-labeled hexadecane, calculating the rate of $^{14}CO_2$ production (r), and converting this rate to a hydrocarbon transformation rate (R) using the following equation:

$$R = r(Sn + A)$$

where Sn was the ambient hydrocarbon concentration and A was the added hydrocarbon concentration in μg/g dry weight of sediment. The rate of $^{14}CO_2$ production (r) was calculated based on zero-order or first-order kinetics, depending on the fraction of the added label that appeared as CO_2 during the incubation period. The equation was based on the assumption that added ^{14}C-hydrocarbons were completely mixed and equilibrated with ambient hydrocarbons in the slurries.

To recover $^{14}CO_2$, acidified samples were purged for 15 minutes with N_2 gas (30 mL/minute) through a Harvey trap containing 15 mL of acidified toluene. The trap effectively scavenged unoxidized or partially oxidized volatile hydrocarbons purged from the sample along with the CO_2. The gaseous stream was then bubbled through a standard liquid scintillation vial containing 10 mL of CO_2-sorbing phenethylamine cocktail. The radioactivity in the vial was counted in a Beckman Model LSC 1800 liquid scintillation counter with automatic quench correction.

All dry weight determinations were obtained for each sediment sample by removing approximately 50 g of sediment and weighing in a tared container. The samples were dried at 90°C for 24 hours, cooled, and reweighed. Sediment dry weights were used to standardize all of the data.

ECOLOGICAL MONITORING

Water samples collected offshore in Cubitainers were transported to the laboratory in Valdez and analyzed for several parameters that might be affected by bioremediation research efforts. Analysis

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included measurements reflecting possible eutrophication, release of oil from the beaches, toxic effects from the fertilizers, and the presence of mutagenic oil residues. Procedures for these measurements are as follows:

Chlorophyll

One-liter water samples were filtered through glass fiber filters, and the filters were extracted with a solution of 90% acetone and 1 N NaOH. After overnight incubation in the refrigerator, samples were centrifuged and the optical density of the supernatant was determined at 750 nm (total absorbance) and 665 nm (chlorophyll a). Phaeophytin was determined by rereading the optical densities after the addition of 10% HCl.

Primary Productivity

Photosynthetic productivity by phytoplankton was estimated by incorporation of ^{14}C-bicarbonate. Plankton samples collected in the field were transported to the Valdez laboratory, incubated in BOD bottles in an outside waterbath, filtered, and frozen. Prior to July 5, 1989, samples were then sent to the U.S. EPA Environmental Research Laboratory (ERL)/Gulf Breeze for analysis using a liquid scintillation counter. Once the liquid scintillation counter was operational at the Valdez laboratory (July 5) primary productivity samples were counted there.

Bacterial Abundance

Estimates of the numbers of bacteria per mL of water in the water column were determined using acridine orange direct counting with fluorescent microscopy (Hobbie et al., 1977). Water samples were filtered through black Nucleopore 0.2 μ pore size filters and stained with buffered acridine orange solution (Fisher Chemical). A minimum of 200 bacterial cells were counted in 5 to 10 grid fields in the microscope.

Bacterial Productivity

The thymidine incorporation method of Fuhrman and Azam (1982) was used to measure bacterial productivity. Triplicate water samples were spiked with 5 μL of ^{3}H-methyl thymidine (1.1 μCi; 2.86 nM final concentration), incubated for 20 minutes and then extracted with 5 mL of cold

10% trichloroacetic acid (TCA). Samples were filtered through 0.22 μm Millipore filters, washed with cold TCA, and the radioactivity on the filter was measured in a liquid scintillation counter.

Caged Mussels

Mussels (*Mytilus edulis*) collected from Tatitlek Narrows, an area not affected by the oil spill, were kept in cages at various sites at Snug Harbor (for ca. 10 weeks) and Passage Cove (for ca. 6 weeks). At each site four cages filled with 25 mussels each were deployed to measure the uptake of petroleum hydrocarbons that might be released into the water column following fertilizer application on the beaches. Mussels were removed at approximately weekly intervals from the cages and sent to EPA/Gulf Breeze for analysis of polynuclear aromatic hydrocarbons (PAHs).

At each sampling, 3 mussels from each cage were sacrificed and the tissues were removed from the shell and frozen. The frozen tissues were returned to the laboratory, where the tissues from all 3 mussels from a single cage were extracted by homogenizing and spiking approximately 20 g of tissue with appropriate surrogates, digested with 6 N KOH at 35°C for 18 hours. The sample was then serially extracted with 3 X 30 mL portions of ethyl ether (Warner, 1976). Extracts were then cleaned up by elution from silica gel columns (SOP #EV89-5, 1989). Concentrated, cleaned-up extracts were then analyzed by capillary column gas chromatography (SOP #EV89-2, 1989). Samples were analyzed for 16 PAHs and total identified and unidentified PAHs. Percent moisture (EPA method 3550) was determined for each sample so results could be expressed on a wet weight or dry weight basis.

Field Toxicity Tests

To characterize the extent to which toxic concentrations might develop during or immediately after fertilizer application to oiled shorelines, a series of toxicity tests were conducted using field water samples and a testing scheme similar to that used to test acute toxicity of industrial effluents.

Water samples were collected at specified intervals before and after application of INIPOL to shorelines in Passage Cove and were sent to the consulting laboratory MEC (Marine Environmental Consultants in Tiberon, CA.) for 48-hour toxicity tests with oyster larvae *Crassostrea gigas*. Tests followed the ASTM (1980) practice for conducting static acute toxicity tests with larval molluscs. The MEC final report to this project specifies test methods and daily observations (MEC, 1989). A variety

of laboratory and field controls were utilized during testing. Endpoints monitored for these tests were larval survival and percentage of larvae that exhibited abnormal development.

One water sample (field control) was collected at the field control site, immediately outside of the test area, just before the initiation of fertilizer application. At the beach where fertilizer was applied (a 100 m stretch of shoreline), water was collected at 0.5 m depth (just above the bottom) immediately offshore. Water samples were collected immediately before fertilizer application (pre-application, which was 2 hours before low tide), following the completion of application (2 hours after low tide), and again after 1 hour, 3 hour, 6 hour, 12 hour, and 18 hour intervals. Sampling stopped at this time in order to return samples for shipping. All water samples were maintained at 4°C until toxicity tests began.

Oyster larvae toxicity tests were conducted with a standard dilution series (100%, 56%, 32%, 18%, and 10%) prepared for each water sample collected after application. Because the salinity of site water was 26 ppt, field samples were adjusted to 28 ppt by addition of 90 ppt brine solution before test dilutions were prepared. The salinity adjustment accounted for approximately 3% dilution and was selected as the minimum change necessary to ensure that salinity was sufficient to sustain normal development of oyster larvae (this dilution was not accounted for in the subsequent reporting of sample concentrations). The same brine was diluted to 28 ppt and tested as a "hypersaline control" to characterize the adequacy of the brine mixture as a test solution. Laboratory seawater was diluted from 32 ppt to 28 ppt and tested as a seawater control.

LABORATORY FLASK STUDIES

Shake Flasks (Exxon)

Flask studies used samples of Prince William Sound water and/or oiled beach material. All flasks were incubated with slow shaking at constant temperature. At each sampling, flask contents were sacrificed and extracted with methylene chloride. Extracts were analyzed as described above for hydrocarbon composition analysis.

For experiments on the effects of different inocula, samples of artificially weathered Prudhoe Bay crude oil (volatiles removed, 30% weight loss, by distillation) (1% by weight) were placed in sterile Bushnell-Haas medium, to provide nitrogen and phosphorus equal to 3.5% and 4.1% by weight

of oil, respectively. This medium was used uninoculated (control) or inoculated with either a 10% inoculum of water from the Alyeska ballast treatment facility or seawater from Prince William Sound. All flasks were incubated at 15°C for 16 days prior to analysis of oil composition. To determine the effects of incubation temperature, flasks were incubated for 38 days at 15° and 5°C prior to analysis of oil composition.

To determine the relative effectiveness of oleophilic fertilizer, INIPOL, at 3, 10, 20, and 50% of the oil concentration, was added to a poisoned (50 mg/L $HgCl_2$) and a nonsterile flask. Extents of oil degradation were compared to flasks receiving water-soluble fertilizer (WOODACE briquettes) in nonsterile flasks at a rate sufficient to produce a mixture of fertilizer and oil with 0.4% added N and 0.09% added P.

Measurements of INIPOL-enhanced oil degradation on rock surfaces was studied using oiled beach material from Prince William Sound that was covered with INIPOL at concentrations approximating 10% of the oil concentration. Untreated oiled beach material and poisoned controls (50 mg/L $HgCl_2$) served as untreated controls.

Respirometric Flasks

Laboratory flask studies were also conducted using analytical respirometry to determine oil biodegradation rates, and GC/FID chromatography to determine changes in oil composition.

Two nutrient formulations, INIPOL and a defined minimal-salts medium (OECD), were compared. Microbial inocula consisted of seawater from Snug Harbor, beach material collected from an uncontaminated beach in Valdez, weathered crude oil from the spill, and indigenous biota from the Alyeska ballast water treatment plant.

The oil was fractionated into the aliphatic, aromatic, and polar fractions using standard silica gel column chromatography. Composition of the aliphatic and aromatic fraction was measured by gas chromatography using flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS), respectively. Samples were collected at 0 weeks, 6 weeks, and 26 weeks (see methods described under oil chemistry).

Respirometry experiments were performed in a Voith Sapromat B-12 respirometer, consisting of a temperature controlled water bath with 12 measuring units, and a recorder for direct plotting of oxygen uptake curves. Each measuring unit comprised a reaction vessel with a CO_2 adsorber, an oxygen generator, and a pressure indicator. Microbial activity created a vacuum in the reaction vessel, and it was recorded by the pressure indicator. Pressure was balanced by electrolytic oxygen generation from the dissociation of copper sulfate and sulfuric acid. The recorder/plotter constructed an oxygen uptake graph automatically.

Design of the respirometry experiments is summarized in Table 4.2. All vessels contained 2 grams of uncontaminated beach material from Valdez and 1000 mL of seawater collected offshore at Snug Harbor. The vessels containing beach material, oil, and INIPOL were charged by first adding the beach material, pouring a measured amount of oil onto the sand, adding the INIPOL to the oiled rocks, and finally filling the vessel with the Snug Harbor seawater. All reaction vessels were mixed with stirring turbines and incubated at 15°C in the dark.

TABLE 4.2. EXPERIMENTAL DESIGN FOR RESPIROMETRIC STUDIES

Reaction Vessel[a]	Oil Concentration (mg/L)	INIPOL Concentration (mg/L)	Alyeska Ballast Water (mL)
V1,V1R	1000	50	-
V2,V2R	300	15	-
V3,V3R	100	5	-
V4,V4R	1000	50	10
C5	-	50	-
C6	-	-	-
C7	1000	50	-
C8	-	50	-

[a] V = Vessel
R = Replicate
C = Control

Flask microcosm experiments were also conducted to provide further support for the respirometric studies. Each flask contained 20 g of uncontaminated beach material but was prepared in the same manner as the respirometric flasks. The experimental design for these experiments is summarized in Table 4.3. Flasks were incubated on a shaker at 15°C.

TABLE 4.3. EXPERIMENTAL DESIGN OF FLASK STUDIES

Flask[a]	Oil Concentration (mg/L)	INIPOL Concentration (mg/L)	OECD[b]	Alyeska Ballast (mL)
F1,F1R	10,000	500	-	-
F2,F2R	10,000	500	-	10
F3,F3R	10,000	-	+	-
F4,F4R	10,000	-	+	10
C1	10,000	-	-	-
C2	10,000	-	-	10

[a] F = Flask
R = Replicate
C = Control

[b] OECD, a defined minimal-salts medium was composed of the following constituents added to provide the specified final concentration (mg/L) in the test solution: KH_2PO_4 (170), K_2HPO_4 (435), Na_2HPO_4 (668), NH_4Cl (50), $MgSO_4.6H_2O$ (45), $CaCl_2$ (55), $FeCl_3.6H_2O$ (2.5). It included the following trace elements added to provide final concentrations ($\mu g/L$) in the test solution: $MnSO_4$ (60.4), H_3BO_3 (114.4), $ZnSO_4.7H_2O$ (85.6), $(NH_4)_6MO_7O_2 4$ (69.4), and $FeCl_3$ EDTA (200). To prevent trace nutrient limitation, either 1 mL/L of a stock yeast extract solution (15 mg/100 mL), or the following vitamins, biotin (0.4), nicotinic acid (4.0), thiamine (4.0), p-aminobenzoic acid (2.0), pantothenic acid (2.0), pyridoxamine (10.0), cyanocobalamine (4.0), and folic acid (10.0).

Biometer Flasks

Aerobic biodegradation of organic substrates results in the consumption of oxygen and the production of carbon dioxide (respiration). Biometer flasks were used to measure stimulatory effects of various fertilizer treatments on the activity of indigenous, aerobic, oil-degrading microbes by measuring accelerated levels of microbial respiration.

Biometer flasks contained 100 g of homogenized oiled beach material from Prince William Sound sieved (<12.5 mm, >4.75 mm diameter, summer 1990; >2.75 diameter, winter 1989/1990), and mixed well to generate homogenized test substrate of uniform oiling.

To simulate conditions similar to Prince William Sound, oiled material was exposed to an artificial tidal cycle consisting of a 12 hour high tide followed by a 12 hour low tide. Rocks were incubated at 15°C in the dark and tidal action was simulated by gentle mixing (75 rpm). To facilitate tidal cycling while maintaining the integrity of the closed system, the apparatus was modified by placing a Teflon tube, fitted with a swivel lock and sealed with a 10 mL hypodermic syringe, through the rubber stopper holding the ascarite trap (Figure 4.3). A 12 hour "high-tide" period was simulated by adding 50 mL of an aqueous solution of sufficient volume to submerge all rocks through the Teflon tube. For "low-tide", a 60 mL hypodermic syringe was connected to the Teflon tube, aqueous solutions were withdrawn, and rocks were incubated for 12 hours. Each high-tide solution was retained and tested for oil residues physically removed from the test systems.

At each tidal change NaOH trapping solutions were sampled to measure microbial respiration rates. Evolved CO_2 was trapped in 10.0 mL of a 0.5 N NaOH solution (prepared with CO_2-free water) located in the side-arm of the biometer flask. For each 12-hour interval, NaOH was removed from the biometer flask and replaced with 10.0 mL of fresh trapping solution. The amount of trapped CO_2 was determined by acidifying NaOH samples (pH<2.5 with 8.5% phosphoric acid) and analyzing headspace gases by gas chromatography. Production of CO_2 was measured for 5 to 7 days, and background CO_2 concentrations were determined for each sampling point. This procedure was repeated to simulate the requisite number of tidal cycles. For certain experiments, a Micro-Oxymax autorespirometer was used to measure both carbon dioxide production and oxygen consumption simultaneously.

Radiolabeled phenanthrene-9-^{14}C (s.a.=13.1 mCi/mmol) of [U]-^{14}C-oleic acid (s.a.=907 mCi/mmol) were used to measure the activity of "INIPOL-degrading" and oil-degrading microflora. Radiolabeled substrates were introduced with the final aqueous high-tide solutions (2.5×10^6 dpm/50 mL) and added directly to the oiled beach material in the biometer flasks. Release of $^{14}CO_2$ was determined by liquid scintillation analysis on duplicate, 1.0 mL samples of the NaOH solutions recovered at 12 hour intervals over a 3 to 5 day incubation period.

Rubber Stopper

Cotton

Ascarite Trap 8 To 20 Mesh

Stopcock

Rubber Stopper

250mL Erlenmeyer Flask

50mL Sterile Seawater

Oiled Beach Material

Incubation Conditions

- Daily Tidal Exchange
- 12 Hours High Tide / 12 Hours Low Tide
- 15° C, Dark
- Agitation - 50 rpm
- Sterile Control

Rubber Stoppers

15 Gauge Needle

50mL Tube

NaOH

Polyethylene Tubing
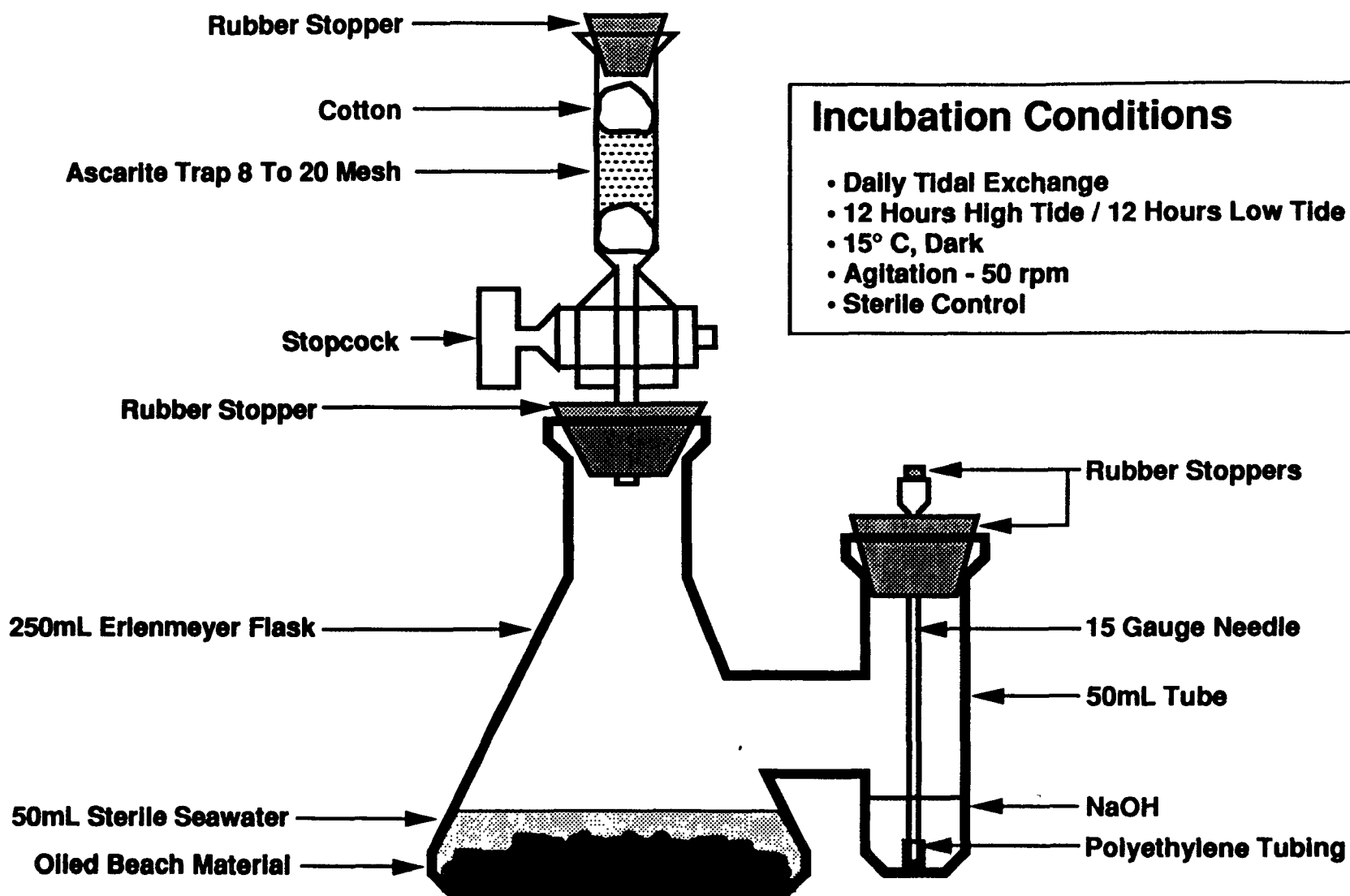
Figure 4.3.   Biometer Flask - "High Tide".

Measurement of Carbon Dioxide

The procedure for measurement of CO_2 in aqueous solutions used a Dohrmann DC-80 carbon analyzer, which was installed, plumbed, and operated according to the low temperature system set-up described in the operator's manual with three exceptions. Since only CO_2 was measured, the reactor reagent was 2% phosphoric acid and not the 2% potassium persulfate/phosphoric acid mixture called for by the manual. Also, the UV lamp was not lit. Finally, the sample was not acidified and sparged prior to injection.

An appropriate amount of sample (approximately 20 μL) was hand injected into the DC-80. The operator's manual provides the information to determine amount of sample to inject. The DC-80 possesses a self-contained pumping system which cycles (at $\geq$ 2.5 mL/minute) reactor reagent past the injection port. When a sample is injected the pH shift (to < pH 2) results in the immediate release of CO_2 from the NaOH. The CO_2 is cycled through the reactor and carried to the Non-dispersive Infrared Detector (NDIR) on a stream of O_2 (zero grade, 200 cc/min). The detector measures the peak as an electrical output and the processor in the DC-80 converts the peak to a parts per million carbon (ppm C) value.

In some tests samples were placed in a biometer flask coupled to the Micro-Oxymax auto respirometer to detect changes in the oxygen and carbon dioxide concentration in the headspace gases of each biometer flask. This was accomplished by cycling the gases through an electrochemical fuel cell to detect oxygen and an infrared spectrophotometer to detect CO_2 at 3 hour intervals. Results were reported as rate of O_2 and CO_2 consumption/production. Following 3 days of respirometric analysis with the Micro-Oxymax, a fourth high-tide solution with radiolabeled phenanthrene was added to each biometer to monitor specific microbial activities.

MICROCOSM STUDIES

Microcosm studies permit the testing of bioremediation concepts under idealized conditions to provide complementary data and information to field demonstration projects. Three types of microcosms were tested: jar, tank, and column.

Jar Microcosms

Experiments to determine the numbers of oil-degrading and oleic acid-degrading microorganisms resulting from the application of INIPOL fertilizer were conducted in chemically clean (I-Chem) jars, each containing approximately 200 g of oiled beach material plus either seawater, artificial seawater, or sodium chloride solution (20%). The artificial seawater was composed of (per liter of distilled water): NaCL (24 g) $MgSO_4.7H_2O$ (1.0 g) KCL (0.7 g) KH_2PO_4 (2.0 g). Na_2HPO_4 (3.0 g), and NH_4NO_3 (1.0 g). The medium pH was adjusted to 7.4 with 1.0 N NaOH following autoclaving. For sterile systems, the oil-contaminated beach material was autoclaved in I-Chem jars. This process removed the water from the oil but did not remove the oil from the beach material. INIPOL application consisted of dripping 3 mL of INIPOL on the beach material surface and allowing the treated material to incubate for 3 hours before filling the jars with the appropriate aqueous phase (about 100 mL). Except for the jar containing unautoclaved seawater, sterile medium (seawater, artificial seawater, or NaCl solution) was used in each microcosm. Subsamples (1.0 mL) were removed at 24-hour intervals for bacterial enumeration. Oleic acid-degrading bacteria were enumerated on oleic acid-containing agar plates supplemented with nitrogen and phosphorus. Oil-degrading bacteria were enumerated by the dilution to extinction technique described under Microbiology in Section 4. After collecting bacterial enumeration samples, the aqueous phase from one set of jars was decanted off and replaced with fresh sterile medium (fresh seawater was added to the nonsterile seawater jar). The decanted solution was frozen for analysis of residual oil components.

Tank Microcosms

Microcosms were constructed onboard the motor vessel AUGUSTINE to simulate field demonstration treatment and untreated control plots within the beaches. Six troughs were used to hold nine 2-gallon polyethylene tanks. A schematic of the microcosm system is shown in Figure 4.4. Twenty-seven of the containers were filled with homogenized oiled mixed sand and gravel (mixed in a large plywood box on the beach) obtained from the beaches in Snug Harbor. The remaining 27 containers were first filled about one-fourth full with the homogenized mixed sand and gravel, and then filled with oiled cobble. The microcosm containers had four 2.5 cm holes in the bottom to allow percolation of the water through the beach material as the troughs filled. Seawater from the harbor was pumped into the troughs, held for 6 hours (high tide) and withdrawn to simulate tidal cycles. The tanks, therefore, remained dry for 6 hours. Within each trough with nine tanks, three replicate tanks were sacrificed at three intervals. These were analyzed to characterize weight and composition of the remaining oil. Intermittent samples were taken for nutrient analyses.
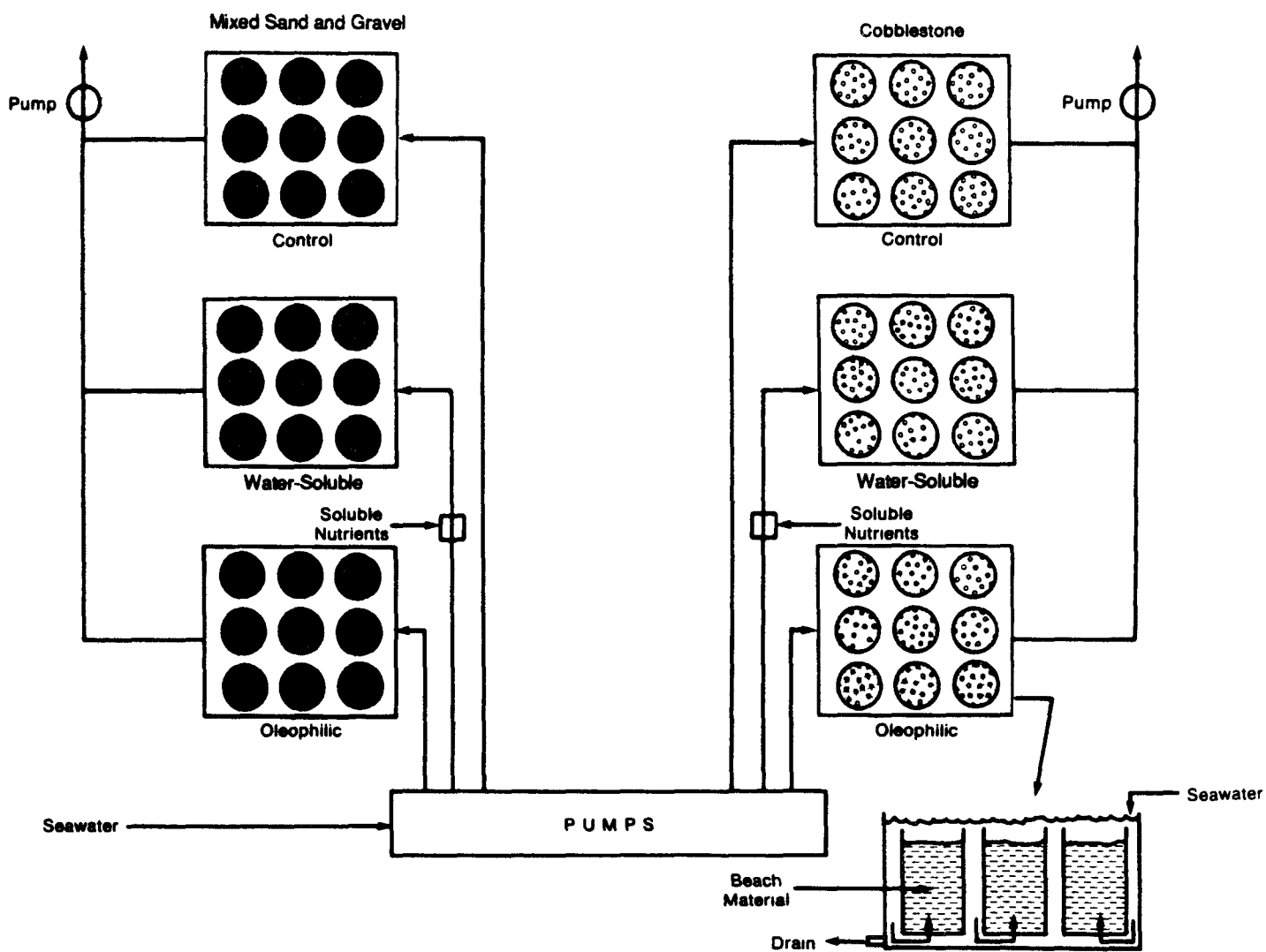
Figure 4.4. Schematic Diagram of the Tank Microcosm.

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Two types of fertilizer were tested in the microcosms. Oleophilic fertilizer (INIPOL) was applied to the microcosms on June 16, 1989 with portable backpack sprayers, in sufficient quantities to coat the exposed surface of the beach material. Eighty IBDU briquettes were placed in a container so that water entering the microcosm flushed over the briquettes. However, because ammonia in the microcosms was never above background concentrations during the first week of operation, the briquettes were replaced with small bags filled with commercial granular fertilizer (N:P:K ratio of 16:5:5, not slow-release), to ensure adequate levels of nutrients were maintained. This approach continuously produced ammonia concentrations around 400 to 700 mg/L at each filling of the microcosms.

Column Microcosms

Microcosms consisted of jacketed chromatographic columns, 450 mm long and 25 mm in diameter (Ace Glassware, Inc., Cat. No. 5821-26). A refrigerated bath/circulator was used to circulate water through the column jackets to maintain a temperature of 15 ± 1.5 °C. A small plug of glass wool was inserted into the bottom of each column and 300 g of oiled beach material from Elrington Island was added. This material contained approximately 6 g of oil per kg and was sieved to between 4.75 and 12.5 mm.

Peristaltic pumps and silicone tubing were used for moving air and water to and from the microcosms. Peristaltic pumps, controlled by a 10-program, microprocessor-based timer/controller, were used to simulate two tidal cycles per 24-hour period. A tidal cycle consisted of: 1) pumping water into the bottom of the microcosm for approximately 2 hours (Figure 4.5); 2) maintaining a "high tide" for an additional 4 hours; 3) draining the column by flushing carbon dioxide-scrubbed air through the top of the column and continuing to purge air from the headspace for 1 hour (Figure 4.6); 4) maintaining "low tide" for 6 hours; and 5) purging air from the column to remove all carbon dioxide formed during low tide (Figure 4.7).

Water from Prince William Sound was passed through a glass fiber filter and air was pumped through Ascarite to remove carbon dioxide. Effluent water from the systems was acidified and the carbon dioxide purged into sodium hydroxide traps for determination of oil mineralization during the high tide phase. Air exiting the microcosms during low tide was passed through other sodium hydroxide traps for assessment of carbon dioxide produced during the low tide phase. Carbon dioxide was assayed using a Dohrmann Total Carbon Analyzer with a Dual Sparging Unit. Effluent water was
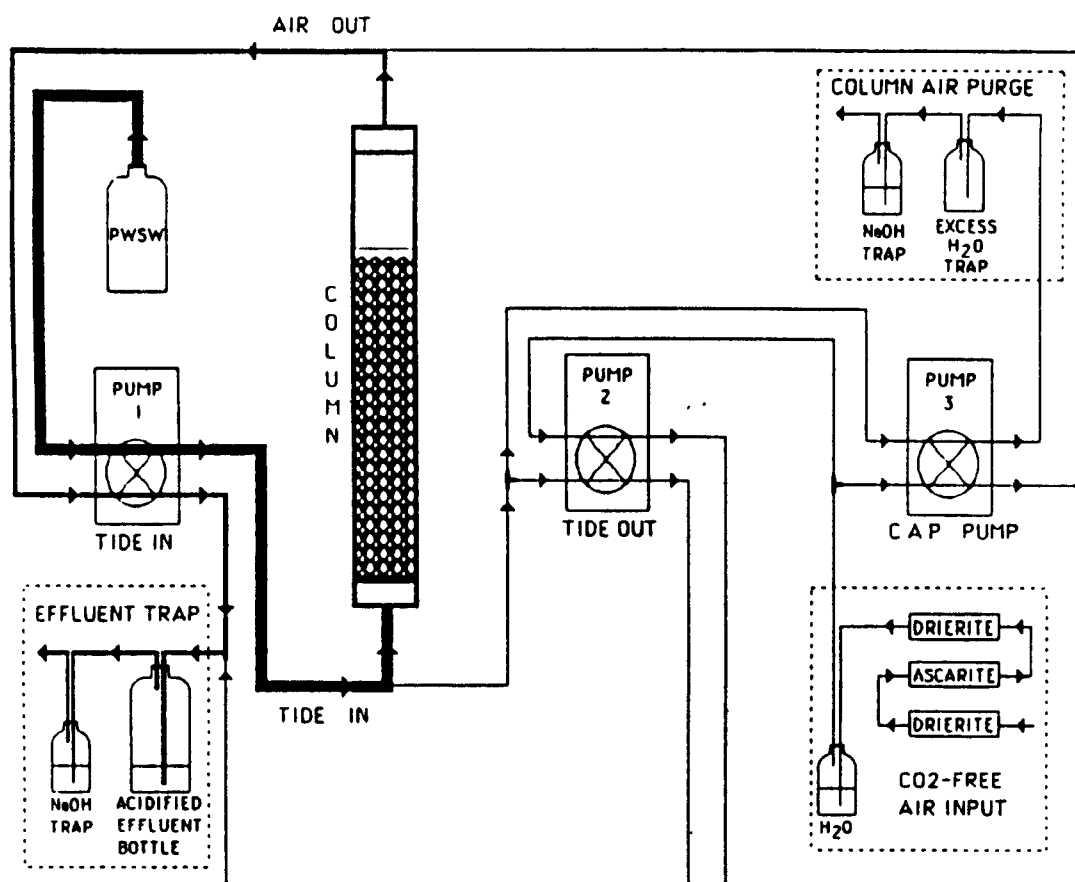
Figure 4.5. Schematic of Flow-Through Column Microcosm - Use of Pump 1 to Fill Columns.

PWSW= Prince William Sound Water

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AIR IN

COLUMN AIR PURGE

NaOH TRAP   EXCESS H2O TRAP

COLUMN

PUMP 1

TIDE IN

PUMP 2

TIDE OUT

PUMP 3

CAP PUMP

PWSW

EFFLUENT TRAP

NaOH TRAP   ACIDIFIED EFFLUENT BOTTLE

TIDE OUT

DRIERITE

ASCARITE

DRIERITE

H2O

CO2-FREE AIR INPUT

PWSW= Prince William Sound Water
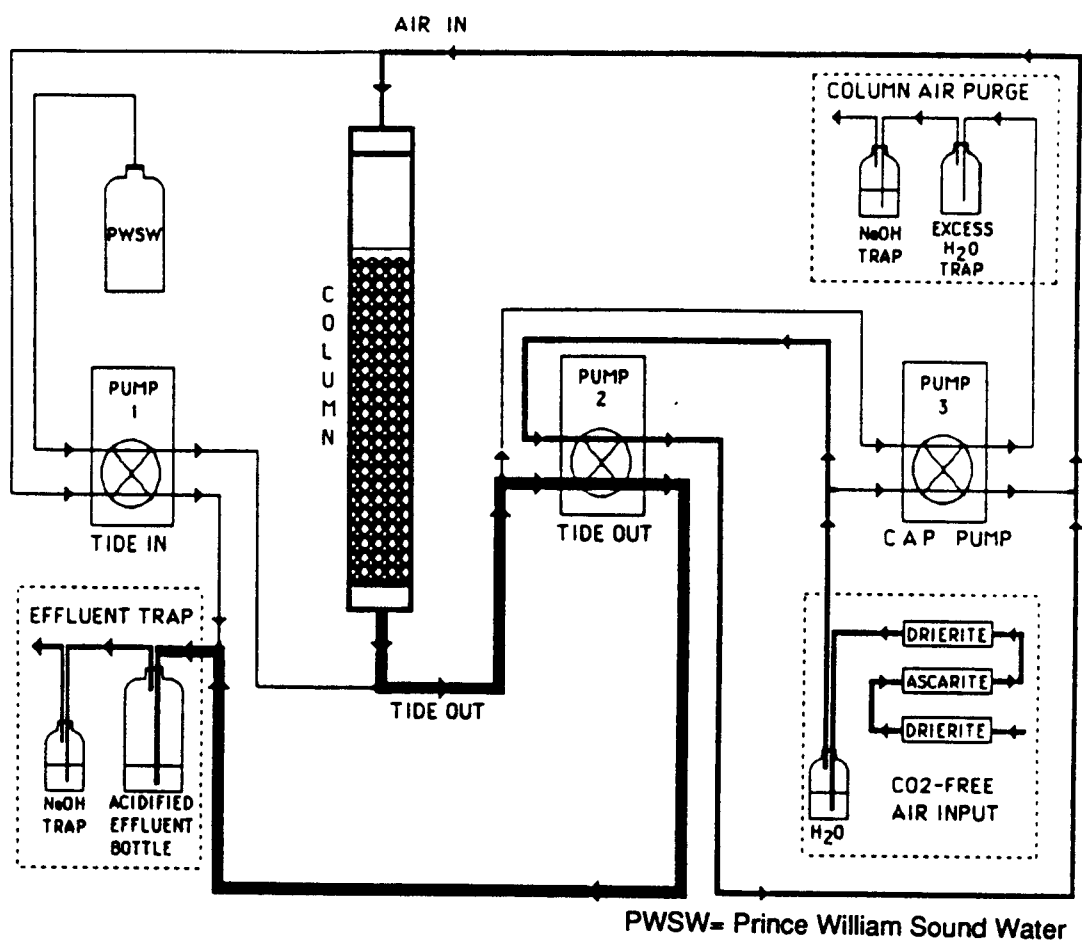
Figure 4.6. Schematic of Flow-Through Column Microcosm - Use of Pump 2 to Drain the Columns.

AIR IN

COLUMN AIR PURGE

NaOH TRAP EXCESS H₂O TRAP

PWSW

COLUMN

PUMP 1

TIDE IN

PUMP 2

TIDE OUT

PUMP 3

CAP PUMP

EFFLUENT TRAP

NaOH TRAP ACIDIFIED EFFLUENT BOTTLE

AIR OUT

DRIERITE
ASCARITE
DRIERITE

H₂O

CO2-FREE AIR INPUT

PWSW= Prince William Sound Water
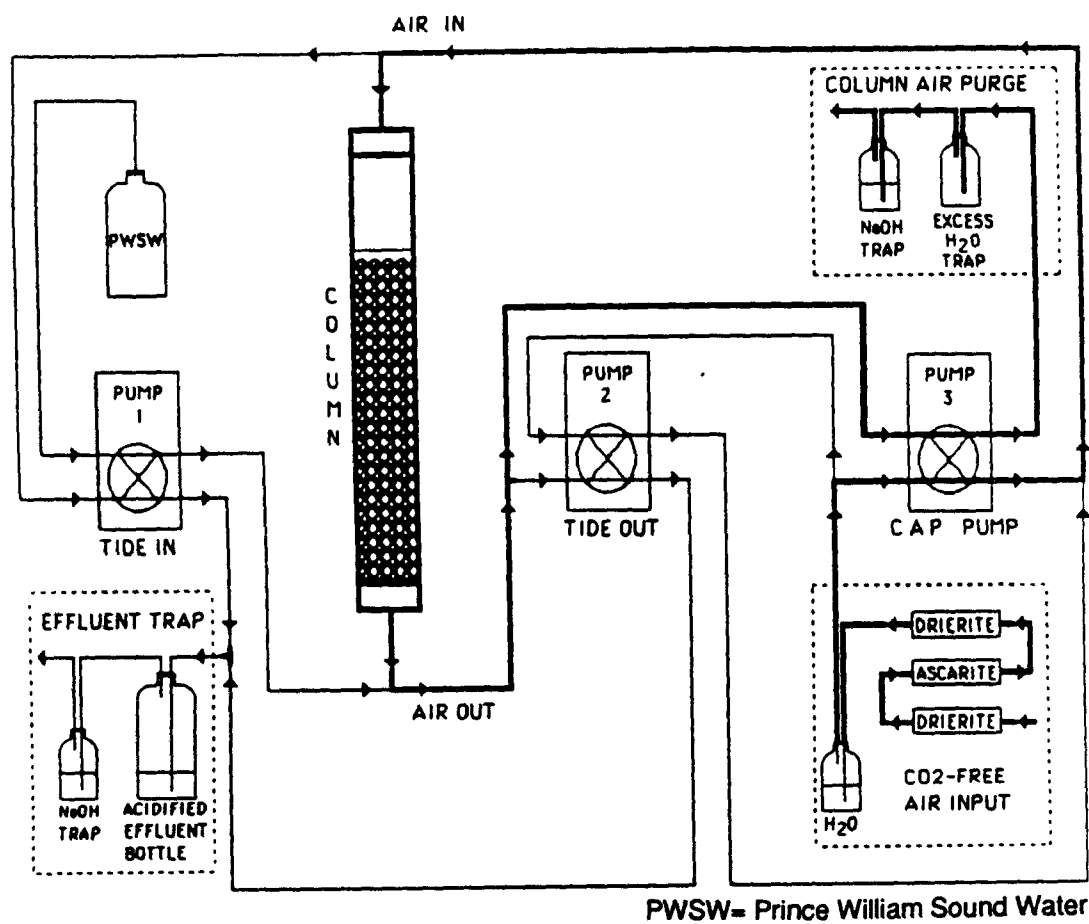
Figure 4.7. Schematic of Flow-Through Column Microcosm - Use of Pump 3 to Purge Air from the Columns.

also analyzed for total organic carbon. Three 300-g samples of oiled beach material were analyzed for oil residue weight at the beginning of the test, and contents of all columns were analyzed for oil residue weight at the end of the test, as a gross index of oil removal.

Carbon from petroleum hydrocarbons may be transformed within the microcosm in one or more of the following manners (Figure 4.8): 1) oil may be mineralized to carbon dioxide and then trapped; 2) organic products may exit the column with the effluent, either as a result of incomplete oil degradation (i.e., organic acids) or bioemulsification and suspension of oil into the water column; or 3) oil may be converted into microbial biomass and stay associated with the oiled beach material. Oil carbon exiting the system as a result of the first two processes should be quantified by the selected procedures (carbon dioxide by sparging into the TOC analyzer and incomplete degradation products and emulsified oil by TOC analysis). No attempt was made to quantify oil converted to biomass, which suggests that information provided by this system may be conservative relative to the overall effects of bioremediation.

Each treatment was tested in duplicate. Two columns containing combusted oiled beach material provided data for normalizing carbon dioxide in the exiting water and air of all other treatments. Two columns containing oiled rock without nutrients provided a control for natural effects without bioremediation. Two columns were treated with a nutrient "bath", consisting of 20 mL of a 35 ppm N, 7 ppm P solution, added with high tide every 4 days (similar to one of the shake-flask tests). Two columns were also treated with a nutrient "sprinkle", consisting of 40 mL of a 175 ppm N, 35 ppm P, solution applied for 2 hours every 4 days. The test was run for a 2-week period.

CHEMICAL EFFECT OF OLEOPHILIC FERTILIZER

The test system designed to address the effectiveness of INIPOL as a potential "rock-washer" consisted of a separatory funnel containing a small amount of oiled beach material. INIPOL was added to the oiled beach material at 5% of the oil concentration and the material was refrigerated at 5°C for 1 hour. Artificial sea water at 5°C was added to cover the beach material, and was again refrigerated at 5°C for 6 hours. The beach material was then drained, and the amount of oil in the water was estimated. A typical test used four washing cycles.
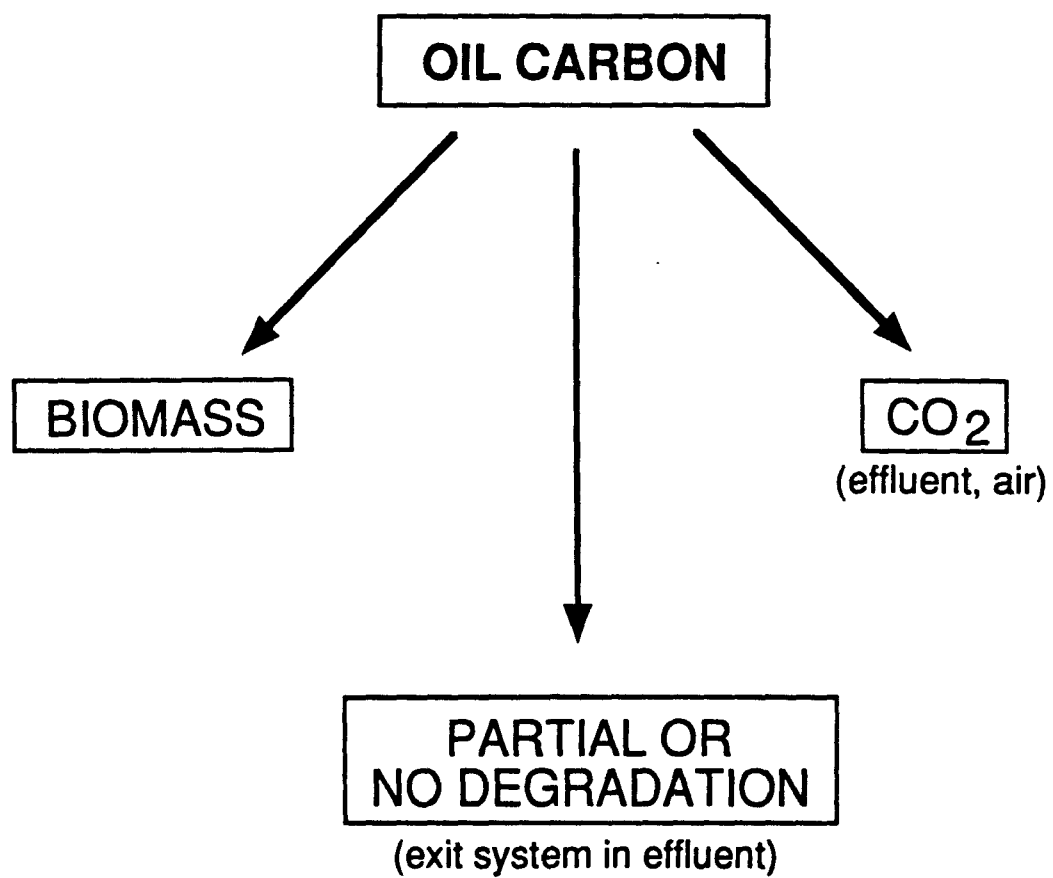
Figure 4.8.   Potential Fate of Oil Carbon in Flow-Through Microcosms.

TOXICITY STUDIES

Acute Tests

Using acute tests to account for worst-case conditions, the oleophilic fertilizer, INIPOL, was tested in seawater alone. Since the fertilizer is very likely to become bound to oil after application to oil-contaminated shoreline, and data generated by the manufacturer show that toxicity of the fertilizer is appreciably decreased in the presence of oil, a second test treatment was selected. This test treatment involved layering of oil on seawater, spraying on fertilizer, mixing, and testing. The oil was also tested alone to provide data for comparative purposes.

Battelle Northwest laboratories conducted acute toxicity tests with silver salmon smolts (*Onchorhynchus kisutch*), Pacific herring (*Clupea harengus passasii*), and bay mussel larvae (*Mytilus edulis*). The oil used in these tests was weathered ANS crude oil collected May 5, 1989, during Exxon Valdez oil spill clean-up operations, from skimmer #81 working west of Disk Island in Prince William Sound. This oil sample and the INIPOL used for testing was supplied by scientists at Exxon Research and Engineering, Annandale, New Jersey. Toxicity testing procedures were adapted from EPA protocols published for testing crude oils and dispersants (EPA, 1984), substituting INIPOL as the dispersant chemical. Tests with mussel larvae followed ASTM standard methods (ASTM, 1988) with minor modifications based on dispersant test methods (EPA, 1984). A detailed methods description can be found in the Battelle data report to the EPA (Antrim and Word, 1989).

EVS Consultants tested threespine sticklebacks (*Gasterosteus aculeatus*), Pacific oyster larvae (*Crassostrea gigas*), mysids (*Mysidopsis bahia*), and pandalid shrimp (*Pandalus danae*) commonly known as rock shrimp. Exxon supplied EVS with samples of the same oil and INIPOL that was tested by Battelle. Stocks of test solutions prepared from oil, INIPOL, and mixtures of oil plus INIPOL were treated as effluent samples and acute toxicity tests were conducted following the EPA-recommended methods of Peltier and Weber (1985). Tests with oyster larvae followed ASTM methods for molluscs (ASTM, 1986). Complete test details are in the EVS final report (EVS, 1990).

Chronic Estimator Toxicity Tests

Chronic estimator toxicity tests with INIPOL and two standard estuarine test species of fish, the inland silverside, *Menidia beryllina*, and the sheepshead minnow, *Cyprinodon variegatus*, were

conducted at the EPA Laboratory in Gulf Breeze. A contract laboratory conducted a parallel test with Menidia. All tests followed Methods for Estimating Chronic Toxicity of Contaminants to Marine Organisms (EPA, 1987). During the studies, INIPOL test solutions were replaced daily in static chambers containing larval fish that were 7 to 10 days old at test initiation. Survival and growth of the fish were monitored as test endpoints for the seven-day test. Results are expressed as the 7-day LC50 (concentration lethal to 50% of the test population), maximum test concentration resulting in no significant lethal effects, and maximum test concentration yielding no significant reduction of fish growth.

Toxicity of INIPOL and its Constituents to Mammalian and Avian Wildlife

Computerized data bases were searched employing a combination of key words that cover data for routine laboratory test species and also incorporate information that might exist for wildlife. Searches were conducted on three databases maintained by the National Library of Medicine's Toxicology Information Program: 1) the Registry of Toxic Effects of Chemical Substances (January, 1990 version); 2) Hazardous Substances Data Bank; and 3) Toxline. A search was also conducted in the TERRE-TOX database maintained at the EPA Environmental Research Laboratory in Corvallis, Oregon. These computerized data bases summarize current and historical information from scientific journals, government reports, industrial reports, books on industrial and chemical safety and hygiene, and published regulations and standards. The information stored in these databases includes acute toxicity, chronic effects, metabolism, and sublethal effects. In addition, two EPA documents on ammonia were reviewed.

The search strategy used the following key words (in both the singular and plural form) to probe for animal toxicity data: mammal, rat, mouse, guinea pig, rabbit, cat, dog, wildlife, bird, avian, shorebird, duck, eagle, and raptor. INIPOL and its chemical constituents were searched by name and their Chemical Abstract System (CAS) number, if assigned. INIPOL was reviewed (no CAS #), as was ammonia (CAS 7664-41-7), aqueous ammonia (CAS 1336-21-6), urea (CAS 57-13-6), 2-butoxy-ethanol (CAS 111-76-2), lauryl phosphate (no CAS # found), tri-laureth phosphate (no CAS # found), sodium lauryl phosphate (CAS 7423-32-7), dodecyl phosphate (no CAS # found). Since there was no toxicity information available for the lauryl phosphate component of INIPOL, lauryl sulfate (CAS 151-41-7, synonyms include dodecyl sulfate) and sodium lauryl sulfate (CAS 151-21-3) were also searched.

MUTAGENICITY TESTS

Soil samples for the mutagenicity studies were collected from Snug Harbor approximately 2 months apart in early June, late July, and early September, 1989. Due to the characteristics of some complex mixtures (e.g., insolubility), the standard assay can sometimes be impractical. The Alaskan oil samples were mixtures that were difficult to test in the standard assay. Therefore, the spiral *Salmonella* assay, a modification of the standard *Salmonella* plate incorporation assay, was chosen for the monitoring of these samples (Houk et al., 1989 and Houk et al., 1991). This assay was chosen because it required less total sample material, did not require solvent exchanged into another solvent such as dimethylsulfoxide, eliminated potential artifacts, and saved labor (Maron and Ames, 1983; Claxton, 1987; and Williams et al., 1988).

To prepare the samples for the assay, the samples were extracted using sonication and dichloromethane. The extracts were filtered through silanized glass wool and concentrated to <100 mL using roto-evaporation. After drying with anhydrous $NaSO_4$, all samples were concentrated or diluted to a concentration of 10 mg/mL (a reference point derived from preliminary testing) and stored in a freezer at -30°C until used for the bioassay.

STABLE ISOTOPES

The samples were collected between three and five months after the oil spill and usually within weeks after fertilizer application. Thus, the food-web structure was documented shortly after the accident, and long-term effects were assessed by comparing these results to monitoring efforts conducted later in the summer.

A variety of biological samples were collected for isotopic analyses from Snug Harbor and Passage Cove. A more limited sampling was also conducted on June 13, 1989 on an uncontaminated beach on Tatitalek Island. Samples included both primary producers and consumers. All organisms were collected from the intertidal zone, and in some cases were collected directly from the treatment areas. After collection, samples were immediately placed in Ziploc bags, frozen over dry ice, and stored in a freezer at -20°C. To prepare for isotopic analyses at Texas A&M University, the samples were thawed, rinsed with copious amounts of distilled water and freeze-dried. The samples were placed in a 50°C oven for 24 hours, then ground in a mortar and pestle, and stored in vials in a desiccator.

A few samples of water-column particulate matter (seston) were also collected from Snug Harbor and Passage Cove. Up to 20 L of water sample were filtered through a precombusted glass fiber filter. Preparation of the filter residue containing seston was similar to the biological samples discussed above. Particulate material in samples from root-feeders were used as subsurface samples. For these samples, a liter of filtrate was collected. Samples for dissolved ammonium were analyzed according to Velinsky et al. (1989).

All samples were analyzed isotopically by a modified Dumas combustion that converts organic carbon and organic nitrogen to CO_2 and N_2 gas for mass spectral analysis (Macko, 1981). Between 3 and 5 g of living tissue were placed in quartz tubes with Cu and CuO, and the tubes were then evacuated and sealed. The tubes were heated to 900°C at a rate of 450°C h-1, kept at 900°C for 2 hours, and cooled to room temperature at a rate of 60°C h-1. The slow cooling cycle ensured that any oxides of nitrogen were decomposed to N_2. CO_2 gas was separated from N_2 gas by cryogenic distillation, and CO_2 and N_2 were then analyzed.

Stable carbon and nitrogen isotope ratios are reported according to the standard formula:

$$\delta X = [(Rsample/Rstandard) - 1] \times 10^3 \text{ ‰}$$

where δX is either $\delta^{13}C$ or $\delta^{15}N$, and R is either $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard for carbon was PDB Belemnite, and the standard for nitrogen was ultra-pure tank nitrogen that was standardized against atmospheric nitrogen.

The reproducibility of the measurement for $\delta^{13}C$ of particulate matter was ±0.2 ‰ with a minimum sample size of 50 μg. For $\delta^{15}N$, the precision was ±0.3 ‰ for particulate samples, and ±0.5 ‰ for NH_4^+. The minimum sample size for $\delta^{15}N$ analysis was 50 μg. Samples were compared from untreated and treated beaches on several dates throughout the summer.

Biological samples were also taken on a weekly basis during and/or after fertilizer application on Disk and Elrington Islands. Representative algae and heterotrophic consumers commonly found on these beaches were chosen for analysis. By choosing a diverse group of organisms from the intertidal zone, we were able to take advantage of specific spatial orientations and examine the range of the effect of fertilizer nitrogen on the food chains. Green, brown and red algae species included *Urospora sp., Fucus disticus*, and *Odonthalia sp.*, respectively. Consumer organisms were selected to

include a comprehensive selection of feeding strategies: barnacles (*Balanus glandula*) are suspension feeders; periwinkles (*Littorina sitlcuma*) and limpets (*Tacetara persona*) feed on organic matter deposited on rocks in the intertidal zone; and whelks (*Nucella emegginata*) and eel blenny (*Anoplarcus purpurceus*) represented consumers from higher trophic levels. Seston, found in beach interstitial and coastal waters, were used as an estimate of stable carbon and nitrogen isotope values in phytoplankton.

For each sample, four organisms were taken from the beaches and stored in coolers until transported back to the laboratory and frozen (approximately 2 hours). Samples were taken from the intertidal zone below the Sprinkler beach at Elrington Island. Samples from Disk Island were taken directly from a test beach treated with CUSTOMBLEN fertilizer (1 kg/m^2). Frozen samples were shipped to Texas A&M University for isotope analyses. To prepare for analysis, samples were thawed, rinsed in deionized water, and dried in an oven at 50°C. All four organisms were ground together using a mortar and pestle, samples were oxidized in quartz tubes with cupric oxide, and N_2 and CO_2 gases were isolated by cryogenic distillation. Ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ were analyzed with magnetic-sector mass spectroscopy.

Well samples were collected from Disk Island, from a beach area that had received approximately 100 g/m^2 CUSTOMBLEN fertilizer granules, and from a well on a beach area that was not fertilized. Samples were also recovered from adjacent cove waters at Disk Island. Some samples were also collected from wells at Elrington Island, and in the adjacent waters. These latter samples are not discussed in this report.

Well sampling was accomplished during the incoming tide by removing 50 L of interstitial beach water from the bottom of wells using a peristaltic pump and filtering through a 1 μ cartridge filter into a 50 L container. This 50-L sample was processed for collection of bacterial concentrates as discussed below. An additional 20 L was collected without pre-filtering in a 20-L collapsible container (Cubitaner), the container was vigorously shaken, and suspended particulate matter (SPM) was concentrated by pushing the sample through a 4.7 cm glass fiber filter (GF/F; pre-heated at 480°C for 2 hours). In turn, part of the filtrate was collected in 1-L Nalgene bottles and stored at -20°C. A 500-mL subsample was then filtered through a 2.5 cm GF/F filter for analyses of particulate organic carbon (POC) and nitrogen (PON). Filters were dried at 50°C in an oven flushed with N_2 gas and were stored in Petri dishes.

Water column samples were collected by passing up to 20 L of water sample (or the volume collected when the flow of water was reduced to a trickle) directly through a 4.7 cm glass fiber filter (GF/F; preheated at 480°C for 2 hours). At selected stations, part of the filtrate was collected in 1-L Nalgene bottles and stored at -20°C. A 500-mL subsample was filtered through a 2.5 cm GF/F filter for analyses of POC and PON. For collection of bacterial concentrates, 50 L of sample were pushed through a 1-μ cartridge filter into a 50-L container and processed as discussed below.

Stable Isotope Microcosm Study

A microcosm experiment was conducted using four treatments of oiled gravel: 1) fertilizer; 2) fertilizer and seagrass detritus; 3) seagrass detritus; and 4) an untreated control. Well-sorted oiled gravel from Disk Island was placed in 20-L Nalgene tanks, which were located in the laboratory at ambient temperature. No attempt was made to limit the availability of light in these containers. Each day 0.2-μ filtered water (did not contain bacteria) was added to the containers to cover the gravel for 12 hours. The water was drained from the bottom of the container and the gravel was then exposed to air for the next 12 hours. The water recovered from the microcosms was filtered through a 1-μ cartridge filter, an aliquot of this filtrate was collected for bacterial abundance (AODC) measurements, and the remaining water was filtered through both 2.5 and 4.7 cm GF/F filters for elemental (POC,PON) and stable carbon ($\delta^{13}C$) and nitrogen isotope ($\delta^{15}N$) analyses. The duration of the experiment was 12 days.

Bacterial Bioassays and Nucleic Acid Concentration

Natural bacteria were incubated in filtered cove or filtered interstitial water samples (Coffin et al., 1989). These water samples were filtered through 0.2-μ cartridge filters, and then incubated with a 1% inoculum of a 1.0-μ filtered fraction from the same water sample. These samples were incubated for 72 hours in the dark to allow enough bacterial growth. At the end of the incubation, the sample was pushed through a GF/F filter to concentrate the bacteria.

Details of the bacterial concentration and nucleic acid extraction procedure are found in Coffin et al., 1990. Briefly, nucleic acids were extracted from concentrates of bacteria following the protocol described by Marmur (1961) with modifications to increase the recovery of nucleic acids (Maniatus et al., 1981). Bacteria were lysed chemically with 0.2% SDS at 60°C for 30 minutes. After lysis, nucleic acids were extracted twice with phenol (distilled and frozen prior to use), twice with a 50/50

mixture phenol/chloroform and twice with chloroform. Nucleic acids were precipitated in ethanol and 2% NaCl at -70°C for 10 minutes, and centrifuged. The pellet was rinsed with cold 70% ethanol and dried in a vacuum oven. Finally, nucleic acid was resuspended in distilled water, and the purity and quantity of the extracted material was estimated by UV absorption at wavelengths of 260 and 280 nm (Maniatus et al., 1981). Nucleic acids were freeze-dried in preparation for the stable isotope analyses.

Ammonium and Nitrate Distillations

The technique for measuring the $\delta^{15}N$ of ammonium (NH_4^+) was reported by Velinsky et al. (1989). After NH_4^+ was steam-distilled from the 0.8-L Labconco distillation flask and trapped on the zeolite sieve, 3 mL of 8 N NaOH and 600 g of Devarda's alloy were added to the distillation flask. The flask was then reassembled into the steam-distillation system and a new zeolite sieve trap was added to the distillate side (Velinsky et al., 1989). Heating tape was wrapped around the distillation flask and the sample was heated to 100°C for 30 minutes to convert the nitrate (NO_3^-) to NH_4^+. After heating, the NH_4^+ was steam-distilled and prepared for isotopic analyses as described in Velinsky et al. (1989). The zeolite with the exchanged NH_4^+ was analyzed isotopically as described below. The precision was $\pm 0.5‰$ for NH_4^+. The precision of our modified NO_3^- technique (± 0.5) was better than that reported by previous work (Kreitler, 1975; Cifuentes et al., 1989) and in the range of the NaOBr method used by Liu and Kaplan (1989).

Isotopic Analysis

Suspended particulate matter (SPM) samples that were analyzed for carbon isotopes were put into glass petri dishes and placed in a glass desiccator with concentrated HCl fumes. After 4 hours the samples were gently dried to remove the acid without loss of labile nitrogen. All samples (SPM, bacterial bioassays, and nucleic acid extracts) were analyzed isotopically by a modified Dumas combustion that converts organic carbon and organic nitrogen to CO_2 and N_2 gas for mass spectral analysis (Macko, 1981). CO_2 gas was analyzed on a Finnigan MAT 251 (Laboratory of Dr. Ethan Grossman, Texas A&M University), and N_2 gas was analyzed on a Nuclide 3-60-RMS. The reproducibility of the measurement for $\delta^{13}C$ of particulate matter was $\pm 0.2‰$ with a minimum sample size of 50 μg. For $\delta^{15}N$, the precision was $\pm 0.3‰$ for particulate samples. The minimum sample size for $\delta^{15}N$ analysis was 50 μg.

ANALYTICAL PROCEDURES

Other Analyses

Particulate organic carbon and particulate organic nitrogen concentrations of bacterial extracts and biological samples were measured on a Carlo-Erba CNS analyzer. Bacterioplankton were counted with the acridine orange direct count technique (Hobbie et al., 1977).

SECTION 5
QUALITY ASSURANCE/QUALITY CONTROL

BACKGROUND

A comprehensive quality assurance program was applied to the Oil Spill Bioremediation Project. The environmental measurements obtained were verified correct and validated for use in this project. The measurement data, as found in the finalized data base, are fully usable for the intended purpose.

The QA philosophy for this project centered on the concept of "quality assistance". Quality Assistance provides help to the research scientist at critical times during a research project. Scientific research quality assurance is very different than regulatory quality assurance. Quality assurance should support a research project, not restrict it. Quality assurance in this case helped the principle investigator with organization, provided advice on what was logistically possible, kept measurement systems in control, and controlled or reported the amount of variability in the total measurement system.

A vital element of the quality assurance program was the preparation of Quality Assurance Plans (summer and winter 1989, and summer and winter 1990). These QA Plans were presented to the scientific researcher as an aid to conduct scientifically sound and acceptable experiments. They were prepared in accordance with the guidelines and specifications provided in 1983 by the Quality Assurance Management Staff of the U.S. Environmental Protection Agency Office of Research and Development. They include a project description containing the details of the project design. The project organization is described and data quality objectives are addressed. Field sampling techniques, sample handling and preparation, sample analysis, data management, and data analysis and reporting are described. Detailed standard operating procedures are contained in appendices to these documents.

Quality assurance objectives are generally defined in terms of detectability, accuracy, precision, completeness, representativeness, and comparability. These objectives are derived from data quality objectives (DQOs) which represent the greatest degree of uncertainty allowable in the data; in other words, the risk associated with making a decision based upon the data. As a research and development program, this project initiated several new developmental techniques and procedures for which discrete DQOs could not be defined prior to operation. Instead, the QA program was designed to

allow both control and assessment of measurement uncertainty during the sampling and analysis phases of the project. Additional information on QA/QC for this project is available upon request from Dan Heggem at the Environmental Monitoring Systems Laboratory in Las Vegas, NV.

QUALITY ASSURANCE/QUALITY CONTROL COMPONENTS

QA Plan

The data collection criteria provided a balance between time and cost constraints and the quality of data necessary to achieve the research objectives. The QA Plans were designed to accomplish the following general objectives:

- Establish the QA and QC criteria to control and assess data collected in the project.

- Document sampling, analytical, and data management methods and procedures.

- Utilize assessment samples and procedures to verify the quality of the data.

- Perform field and laboratory on-site audits to ensure all activities were properly performed and any discrepancies were identified and resolved.

- Evaluate the data and document the results.

It was necessary to identify both qualitative and quantitative estimates of the quality of data needed by the data users. Guidelines established by the EPA Quality Assurance Management Staff (Stanley and Verner, 1985) encourage data users to clearly identify the decisions that will be made and to specify the calculations, statistical and otherwise, that are applied to the data.

The raw data were collected during the two major operational phases of the project: sampling and analysis. A certain amount of data measurement uncertainty is expected to enter the system at each phase. Grouping of the data, such as by beach plot configurations, also increases uncertainty. The sampling population itself is a source of confounded uncertainty that is extremely difficult to quantify. Generally, DQOs encompass the overall allowable uncertainty from sample measurement and from the sampling population that the data users are willing to accept in the analytical results (Taylor, 1987). Due to the many confounded sources of uncertainty, overall DQOs for the project were not defined.

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The QA Plan focused on the definition, implementation, and assessment of measurement quality objectives (MQOs) that were specified for the combined sampling and analysis phases of data. The MQOs are specific goals defined by the data users that clearly describe the data quality sought for each of the measurement phases. The MQOs are defined according to the following six attributes:

- Detectability -- the lowest concentration of an analyte that a specific analytical procedure can reliably detect.

- Precision -- the level of agreement among multiple measurements of the same characteristic.

- Accuracy -- the difference between an observed value and the true value of the parameter being estimated.

- Representativeness -- the degree to which the data collected accurately represent the population of interest.

- Completeness -- the quantity of data that is successfully collected with respect to the amount intended to be collected, as specified in the experimental design.

- Comparability -- the similarity of data from different sources included within individual or multiple data sets; the similarity of analytical methods and data from related projects across regions of concern.

The project MQOs are established on the basis of the selection of appropriate methods to obtain the data. The MQOs are reviewed by individuals familiar with analytical methods. If the measurement quality goals cannot be met during the course of the project, the actual level of quality is used to reassess the intended use of the data. A lower than desired attainment of data quality could require different approaches in data analysis or modifications to the levels of confidence assigned to the data. The initial MQOs for oil chemistry and nutrient analysis are presented in Tables 5.1 and 5.2.

Design Characteristics

To control and assess data quality, QA and QC samples are incorporated into the measurement system. The oil spill project's experiments and analyses incorporated a number of these samples into their measurement systems. The QA program was not optimal to assess all types of measurement certainty in this project because it was a research project.

TABLE 5.1 ANALYTICAL LABORATORY WITHIN-BATCH MEASUREMENT QUALITY
OBJECTIVES FOR BEACH SUBSTRATE

Parameter	Method	Reporting units	IDL[b]	Precision	Accuracy	Completeness
Extractable oil	gravimetric	mg/kg substrate	25 ppm	30% RSD	80-120%	80%
C8 through C32	GC-FID	%RT	NA	1.0% RSD	NA	80%
C8 through C32	GC-FID	Response Factors	NA	25% RSD[a]	80-120%	80%
C8 through C32	GC-FID	μ g/g	250 ppm[b]	30% RSD	80-120%	80%
Aromatic HC	GC-FID	μ g/g	5-25 ppb[c]	25% RSD	65-135%	80%
nC18:phytane	GC-FID	ratio	NA	10% RSD	80-120%	80%
nC17:pristane	GC-FID	ratio	NA	10% RSD	80-120%	80%

[a] Response factors for nC17, pristane nC18, phytane: 25% RSD initial calibration; 40% for other n-alkanes
[b] 250 ppm in total extractable material (μ g/mg extractable material); 100 ppb in beach substrate μ g/kg of beach substrate
[c] Water 5 μ g/L; sediment 25 μ g/kg

TABLE 5.2 ANALYTICAL LABORATORY WITHIN-BATCH MEASUREMENT
QUALITY OBJECTIVES FOR QA/QC SAMPLES FOR NUTRIENT ADDITIONS

Parameter	Method	Reporting units	IDL[b]	Precision	Accuracy	Completeness
NH_3-N	SPEC	μ M0.1	15%	90-110 %	80%	
NO_3-N	SPEC	μ M0.05	15%	90-110 %	80%	
NO_2-N	SPEC	μ M0.01	15%	90-110 %	80%	
PO_4-P	SPEC	μ M0.03	15%	90-110 %	80%	

[a] Instrument readings that have been converted (where necessary) to calculated reporting units
[b] Instrument detection limit (method-specific) in reporting units

Quality Assurance Samples

QA samples are samples known to the QA staff but are either blind or double blind to the analytical laboratory. Blind and double blind samples have concentration ranges that are unknown to the analysts, but a double blind sample cannot be distinguished from a routine sample (Taylor, 1987). These samples provide an independent check on the QC process and can be used to evaluate whether the MQOs have been met for any given run or batch, or for all batches (e.g., overall measurement uncertainty). Important characteristics of the QA audit samples include their similarity to routine samples in matrix type and concentration level, and their homogeneity and stability. Every QA sample has a specific purpose in the data assessment scheme. Single blind QA samples were incorporated into all nutrient analyses. Five concentration levels were used to cover the expected ranges of both field- and laboratory-generated samples.

Quality Control Samples

To consistently produce high quality data, the laboratory was required to analyze certain types of QC samples known to the laboratory staff that could be used by the analysts to identify and control analytical measurement uncertainty. Each QC sample has certain specifications that must be met before data for the parameter or analytical run are accepted. The QC samples were nonblind samples to assist the laboratory in meeting analytical MQOs. The QC samples were analyzed by the laboratory and permitted assessment of the accuracy of the physical and chemical analysis.

Replicate Samples - Disk Island and Elrington Island

Two or three sampling baskets were collected from each beach. Initially, triplicate oil chemistry analyses and triplicate biometer tests were conducted on subsamples from each basket. Thereafter, one basket was analyzed for oil chemistry in triplicate, and single oil chemistry analyses were performed on the remaining baskets. One biometer test was conducted on a subsample from each of the three baskets. Four wells were located on each beach at Elrington Island for collection of nutrient samples; these four samples represented replicates for nutrient analyses. The standard deviation among replicates was used to estimate field variability, and 95 percent confidence intervals were used to estimate the range of confidence in the mean population estimate (accuracy). Examples of control charts for nC18 and the nC18/phytane ratio are located in Figure 5.1 and 5.2.
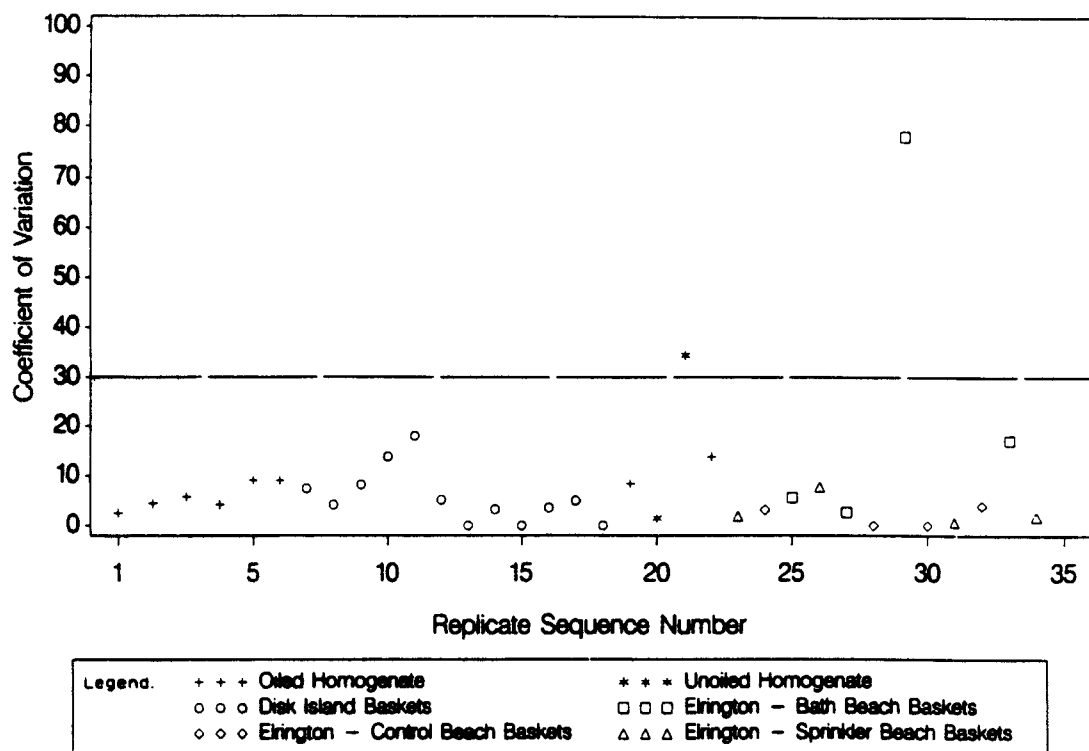
Figure 5.1. Control Chart for nC18 for Disk Island and Elrington Island.
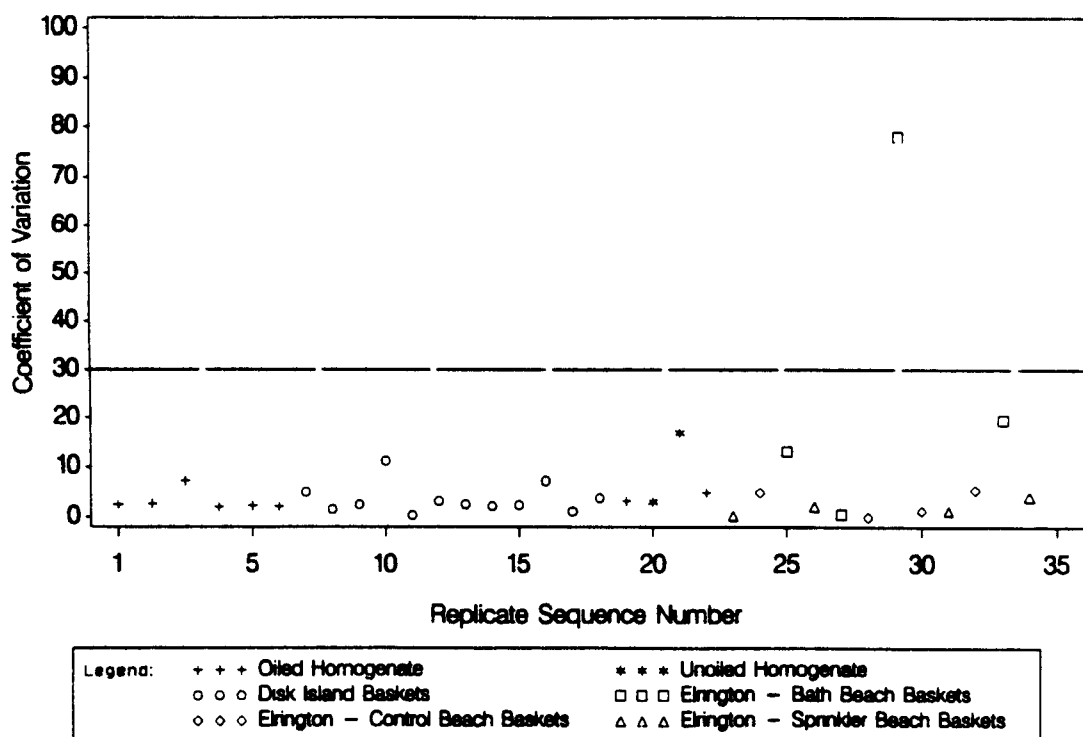
Figure 5.2. Control Chart for the nC18/phytane Ratio for Disk Island and Elrington Island.

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Analytical Duplicate/Triplicate

A duplicate subsample of a routine sample extract (oil chemistry) was selected as the analytical duplicate sample and was used to ensure that within-run MQOs were being satisfied. In the summer of 1989 duplicate oil chemistry was performed on the same extracts. In the summer of 1990, however, replicate extracts were taken from the same sample. In some cases in 1990 triplicate oil chemistry analyses were conducted. Analytical duplicates were also used in nutrient, carbon dioxide, and TOC analyses. One laboratory split was measured in each analytical run. Precision was calculated as the relative standard deviation (RSD, coefficient of variance):

$$RSD = std. \ dev. \ / \ mean$$

Nutrient analysis precision was calculated as the relative percent difference of the duplicate samples (RPD):

$$RPD = | R - D | / \overline{X} \ x \ 100$$

An example of a control chart of nutrient analysis precision for phosphate conducted during the summer of 1990 is shown in Figure 5.3.

Field Audit Blank Sample

The field audit blank (FAB) sample was sent to the sampling crews by the QA staff and was handled using the same procedures as routine samples. The FAB for nutrients consisted of 3 percent NaCl in distilled water. The FAB was used to identify system contamination stemming from sampling and laboratory operations. Pooled data from all FAB samples provided an estimate of the system detection limit (SDL), which was calculated as three times the pooled standard deviation of the FAB samples. FABs were done at Snug Harbor and Passage Cove for the oil chemistry analysis. These samples consisted of solvent extracted non-oiled beach material collected at a site not affected by the Valdez oil spill. An example of the analysis of these blank samples for nC18 is reported in Figure 5.4.
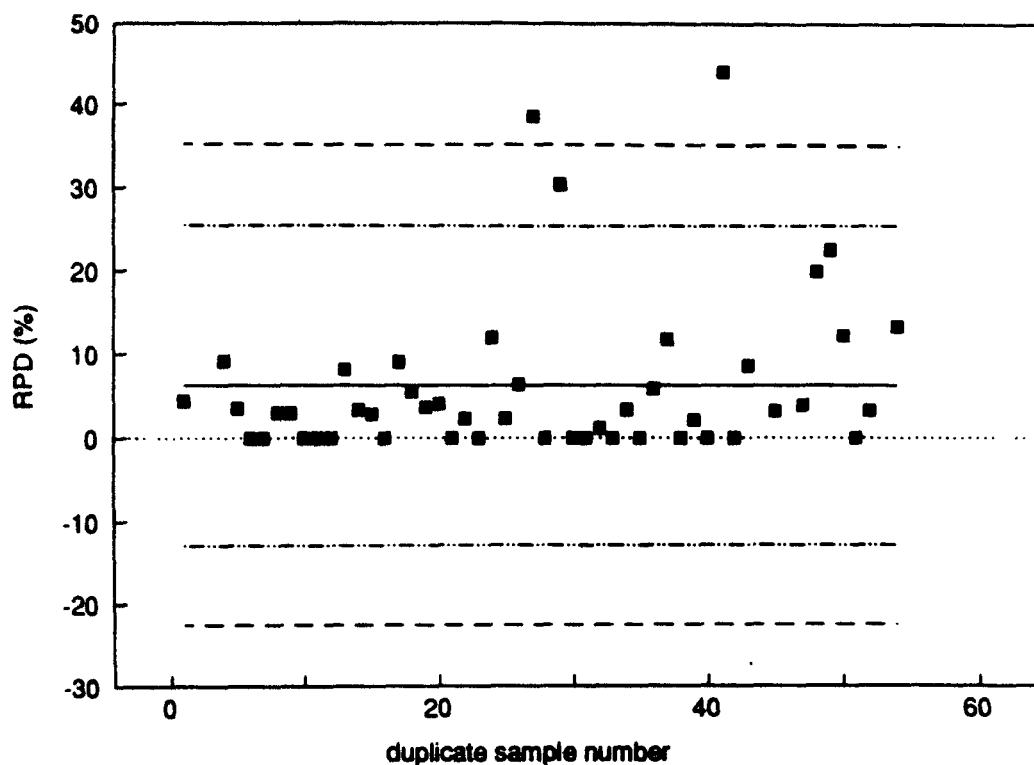
Figure 5.3. Control Chart of Nutrient Analysis Precision for Phosphate During the Summer of 1990.
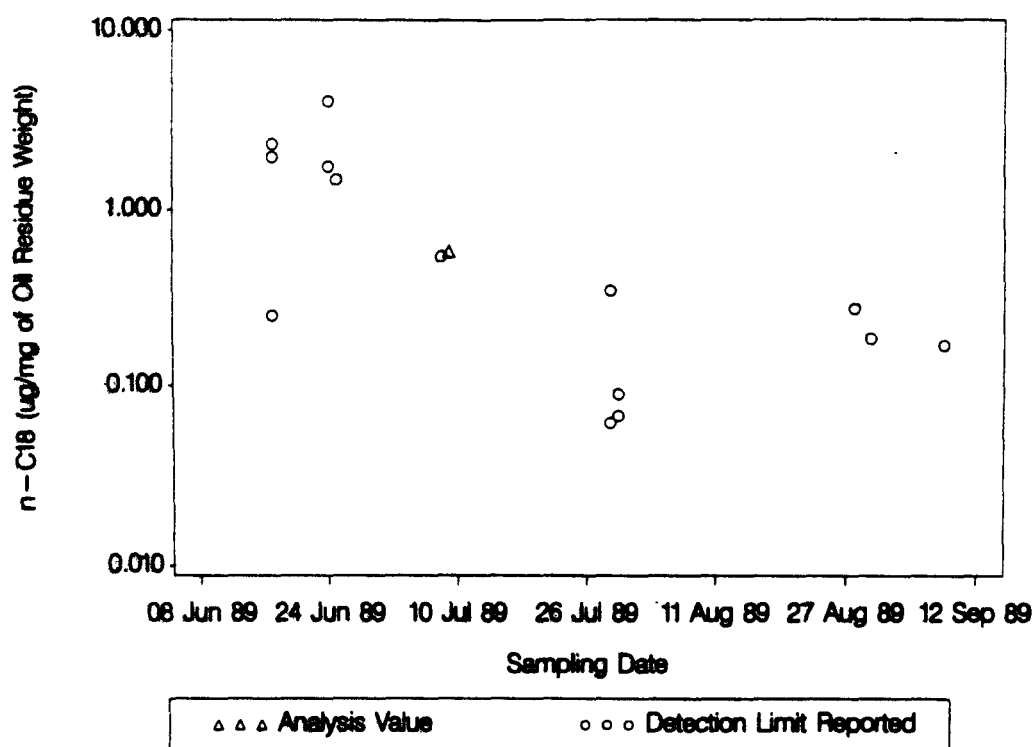
Figure 5.4. Field Blank Analysis for nC18 Over Time for Snug Harbor.

Reagent Blank

A reagent blank consists of all reagents in the same quantities used in preparing a routine sample for analysis. It is subjected to the same procedures as a routine sample, including digestions or extractions. The blank verifies the absence of contamination in the reagents and laboratory sample preparation procedures. The concentration of the reagent blank should be less than or equal to the instrument detection limit (IDL). Any reagent blank exceeding the IDL should be investigated to determine and eliminate the source of contamination. One or more reagent blanks were included in each analytical run for nutrient analyses. For oil chemistry extractions, one or more reagent blanks were initially prepared and analyzed; if no contamination was observed, reagent blanks were prepared only when a reagent lot was changed.

Biometer Test Blanks - Passage Cove, Disk Island, Elrington Island

Two types of blanks were included in each biometer test. One flask contained only air to detect changes in atmospheric oxygen or carbon dioxide. The second blank was a formaldehyde-treated flask. The formaldehyde killed any microorganisms in the flask, creating a laboratory "reagent" blank.

Quality Control Check Sample

A quality control check sample (QCCS) is a standard of known concentration used to verify the calibration curve during sample analysis. The QCCS may be prepared by the analyst or obtained commercially. If purchased, the QCCS should be from a different source than the standards used to generate the calibration curve. The concentration of the QCCS should be in the midrange of the calibration curve or in the midrange of the expected concentration of the routine samples. Values obtained from repeated analyses of the QCCS are plotted on control charts. Examples of control charts for the nC17/pristane and nC18/phytane ratios are shown in Figures 5.5 and 5.6. Control limits are initially set at ± 10 percent until sufficient data are collected to establish warning limits (95 percent confidence interval) and control limits (99 percent confidence interval). A single value outside the control limit or two consecutive values outside the warning limits initiates corrective action (i.e., instrument recalibration and reanalysis of all samples analyzed since the last acceptable QCCS value). The QCCS values are used to assess precision and accuracy of the data.

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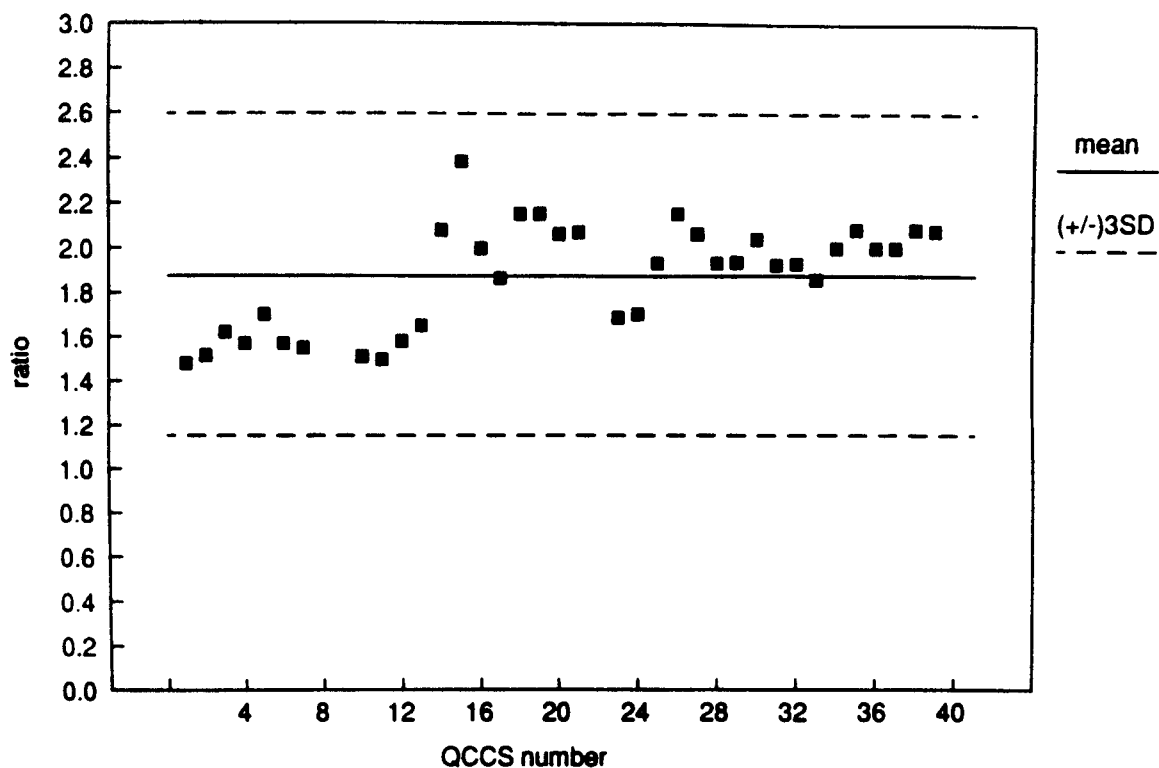
Figure 5.5. Quality Control Check Sample Control Chart for the nC17/pristane Ratio for the Summer of 1990.
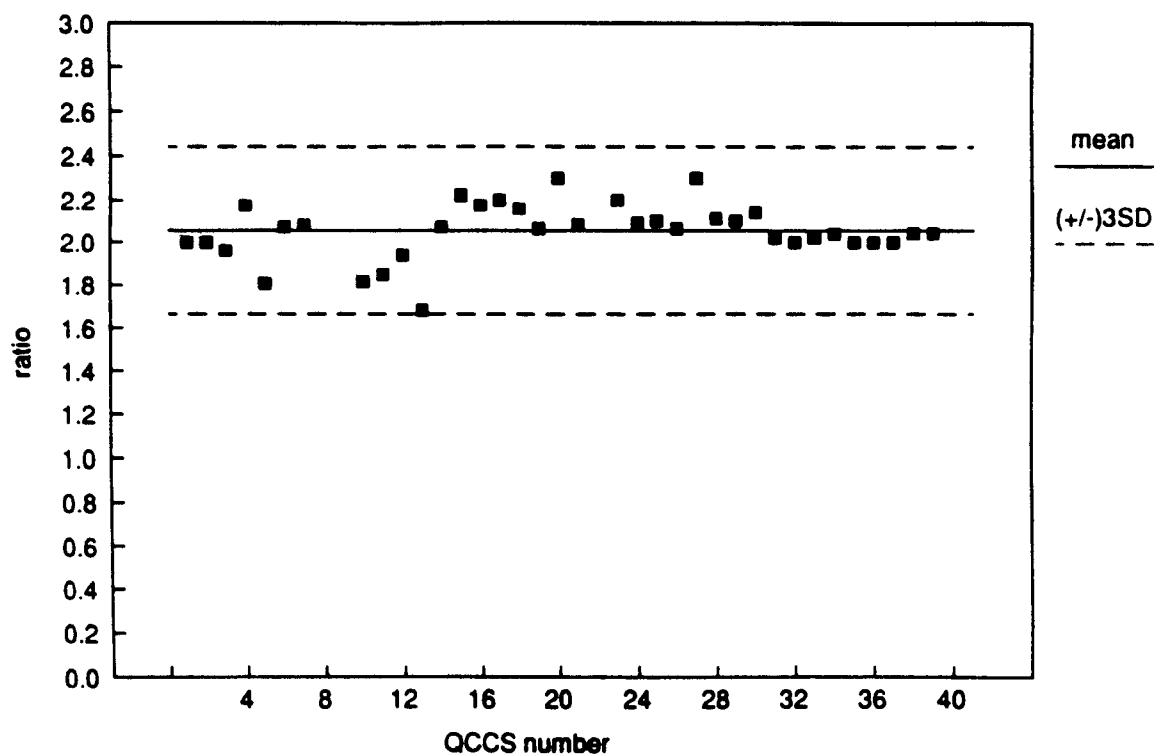
Figure 5.6. Quality Control Check Sample Control Chart for the nC18/phytane Ratio for the Summer of 1990.

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Unweathered Prudhoe Bay crude oil was used as the QCCS for oil chemistry analyses. One QCCS was analyzed with each group of 10 samples, in addition to QCCS analysis at the beginning and end of each analytical run. For nutrient analysis, a volume of an appropriate concentration of standard solution for each nutrient was analyzed as the first sample, after every 10 samples, and as the last sample in each analytical run. For carbon dioxide and TOC measurements, a standard solution in the midrange of the sample concentrations was analyzed as the first sample, after every 15 samples or midbatch, and as the last sample in each analytical run.

Detection Limit QCCS

The detection limit QCCS (DL-QCCS) is a sample containing the analyte of interest at a concentration 2 to 3 times the IDL. The purpose of the DL-QCCS is to eliminate the need for formally determining the IDL for every analytical run. A DL-QCCS was included in each analytical run of nutrient samples. Warning and control limits were established at 2 times and 3 times the standard deviation of the nominal value of the DL-QCCS.

Matrix Spike

A matrix spike consists of the analyte of interest at a concentration approximating 10 times the detection limit. The spike recovery should be 90 to 110 percent of the known value of the spike. If spike recovery is not within this range, the problem should be corrected prior to continuing analysis of routine samples. Two matrix spike samples were included in each analytical run of nutrient samples.

Calibrations

Calibration is the establishment of a relationship between standards of known values and the recorded output of a measurement system. The concentrations of the standards should bracket the expected range of routine sample concentrations. Generally, three to five standards covering the linear range of the particular measurement system are recommended, including one "zero concentration" or detection limit standard. Three-point calibrations were performed daily for nutrient, carbon dioxide, and TOC sample analyses and two-point (zero and 100) calibrations were performed every 9 hours for oxygen and carbon dioxide monitors used in the biometer tests. For the gas chromatograph an initial three-point calibration was performed; thereafter a single-point check was

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performed daily. The three-point calibration was repeated if the single-point check fell outside limits. Additionally, all balances were checked weekly with a minimum of three "S" class weights. A single weight near the weight of the material to be weighed or in the midrange of the balance was checked daily.

Instrument Detection Limits

The IDL is generally defined as 3 times the standard deviation of 7 to 10 nonconsecutive blank analyses. The IDL is formally determined prior to initiation of sample analysis, at periodic intervals thereafter, and following any major disruption of the instrument (repair, move, etc.). A DL-QCCS eliminates the need to formally determine the IDL on a daily basis. The IDL was determined for nutrient analyses and oil chemistry.

Assistance Audits

An important component of the Quality Assurance Program was the use of assistance audits. An assistance audit places the proper quality management resources in the right place at the right time. It was a key concept in the implementation of the Quality Assurance Program. Rather than waiting until the end of a project and assessing the quality of the data, the assistance audit centers on improving the quality of the data on a "real time" basis. The goal of the assistance audit program was to provide data which will meet the needs of the program. An Assistance Audit should give workers a sense of Quality Program "ownership"; give workers a direct way to address corrective actions; and gives the Quality Manager a quick and easy way to implement QA/QC measures.

A summary of Assistance Audits conducted for the Oil Spill Bioremediation Project is located below:

Audit: Field Sampling Activities
Date: June 8 and June 11, 1989
Auditor: Mike Papp

Summary: SOPs, sampling design and QAP were followed. Equipment, supplies, and crew training were all sufficient for the task.

Corrective Actions: No corrective actions were required.

Audit: On-Site Assistance Audit Sediment Oil Chemistry SAIC Laboratory Kasitsna Bay, Alaska
Date: June 10 and 11, 1989
Auditor: Werner Beckart

Summary:

- Laboratory personnel were well educated, had adequate experience and were dedicated to their work.
- Good and adequate instrumentation and equipment were available.
- The QA/QC practiced at the laboratory was adequate.

Corrective Actions: Acquire more freezer space.

Audit: Microbiology
Date: June 12, 1989
Auditor: Linda Stetzenbach

Summary: Great care must be used in the interpretation of the microbiology data gathered from this Project.

Corrective Actions:

- Review the Most Probable Number determination procedures.
- Review the method of detection of positive tubes in the MPN procedure.
- Analyze the cobble beach material.
- Process the beach material in a timely manner.
- Consider limited assay methods.
- Review the data analysis procedure.

Results: John Rogers addressed each corrective action in a letter to Dan Heggem dated July 11, 1989.

Audit: On-Site Assistance Audit SAIC Nutrient Laboratory Kasitsna Bay, Alaska
Date: July 20, 1989
Auditor: Dan Hillman

Summary:

- The QA/QC in this laboratory exceeded the requirements in the Project QA Plan.
- All QC data examined were acceptable.
- The use of Control Charts was discussed with Lab personnel.
- Field or lab blanks were not included with the routine sample batches.
- The use of consistent Instrument Detection Limits and dropping Reporting Limit was recommended.
- The lab personnel were highly motivated and dedicated to achieving QA requirements of the Project.
- Lab performance was outstanding.

Corrective Actions:

- Consider the use of Control Charts.
- Use consistent Instrument Detection Limits.
- Consider dropping the use of Reporting Limits.
- Include field and lab audit blanks in routine sample batches.
- Investigate automated methods and implement if possible.

Audit: SAIC Oil Analysis Laboratory at Kasitsna Bay, Alaska
Date: June 27 - 30, 1989
Auditor: E. Neil Amick

Summary:

- Facilities, equipment and personnel were present to provide quality data.
- Sample tracking and documentation procedures were reviewed.
- Analytical methods were observed.
- Data from QC and routine samples were audited.
- Data produced were of good quality and met the objectives of the study.
- Some deviations from the QAP and SOPs were found.

Corrective Actions:

- Send 10 routine oil extracts and 3 Prudhoe Bay oil audit samples to EMSL-LV for chemical analysis.
- Follow the recommended procedures in the QAP concerning the use of QCCS samples.

Audit: Assistance QA Review of the Ecological Monitoring Program
Date: July 21 - August 9, 1989
Auditor: Jim Pollard

Summary:

- Field sampling reviewed; some early data acquisition problems were resolved.
- QA Plan protocols for chlorophyll processing procedures were in error. The QA Plan needed to be corrected.
- Tracking and documentation systems were reviewed and found to be reliable. Corrections were made in the data base format to ease data analysis and interpretation.
- The design for estimation of measurement error needed to be changed to include a different type of duplicate sample. Field splits vs lab duplicates.
- The field replicate procedure was reviewed.
- A synthetic performance audit sample for nutrient analysis was suggested.
- A flow diagram for ecological monitoring was developed.
- A laboratory QC procedure for chlorophyll *a* measurement was developed.
- A post data entry verification procedure for chlorophyll *a* measurement was developed.
- Data entry and bench sheet generation procedures for chlorophyll *a*, ^{14}C primary production, and bacterial production were developed.

Audit: Cincinnati Nutrient Lab Assistance Audit
Date: November 2, 1989
Auditor: Dan Hillman

Summary:

- Personnel were experienced and suitable for the work.
- Laboratory facilities were adequate.
- Standard methodology was followed with minor modifications necessary due to the sea water matrix.
- All required QA/QC was performed.
- Detection limits were not determined.

Corrective Actions: Alaskan lab and Cincinnati lab should not be compared due to detection limit differences. The Cincinnati lab raw data may be reanalyzed to determine detection limits. A recalculation of Cincinnati data which was less than 0.2 ppm will result in comparable detection limits.

Audit: On-Site Assistance Audit Sediment Oil Chemistry SAIC Laboratory San Diego, CA
Date: November 3, 1989
Auditor: Neal Amick

Summary:

- The results from the SAIC San Diego lab should be directly comparable to the Kasitsna Bay lab.
- Personnel were appropriate for the work.
- The laboratory facility was suitable.
- The quality control and the sample analysis met or exceeded those required in the QAPP.

Corrective Actions: A few deviations from the methods as written in the QAPP were noted. These were the same changes as noted in the Kasitsna Bay, Alaska audits and were included in the next revision of the QAPP.

Audit: On-Site Audit of the Nutrient Laboratory Operations at Valdez, Alaska
Date: August 8, 1990
Auditor: Mike Papp

Summary:

Facilities were small and cramped. Areas were generally well organized and well maintained to maximize efficiency of available space. Safe storage of chemicals and inadequate freezer space were areas of concern. Archived samples were stored in freezer facilities in the microbiology laboratory.

A complete inventory of equipment and materials was not done. In general, equipment and materials were adequate, both in quantity and condition, for the needs of the project. Analysts record bench data could have been recorded neater.

The detection limit QCCS was not run throughout the project. Therefore, no documentation of the instrumental detection limit was available on a batch basis. It was recommended during the audit that detection limits be formally determined. This was done immediately after the audit.

Recommendations and Corrective Actions: Recommendation of the audit included determination of the instrumental detection limits and implementation of a procedure to guard against transcription error during computer data entry. Both of these recommendations were implemented. A review of performance audit data undertaken by the auditors revealed several problems. First, variability of the results was greater than anticipated. Second, estimates of the audits required that the same audits be used for both nitrite and nitrate analyses; audits for remaining nitrate analyses were scheduled to ensure this was done. Finally, ammonia loss was observed in the high concentration audit material. The remaining aliquots of this audit were removed and destroyed. Following the audit, on-going review of the data by the QA staff and data base manager revealed at least two incidents in which dilution factors appeared to contribute to poor results, either because they were incorrectly recorded or excessive dilution resulted in readings at the low end of the calibration curve. No provisions for prevention of these types of errors was included in the laboratory procedures or the QA Plan. It was recommended that a standard procedure be developed or checks implemented to guard against errors related to sample dilution.

Audit: On-Site Audit of the Microbiology Laboratory Operations at Valdez, Alaska
Date: August 9, 1990
Auditor: Mike Papp

Summary:

Facilities were small and cramped, but the lab was well organized and well maintained to provide maximum efficiency within the constraints of cramped space. Efforts were made to provide safe storage of chemicals. Temperatures of the refrigerators and freezers were not recorded daily. It was recommended that logs be maintained for each refrigerator and freezer unit. Hazardous materials used in the laboratory included acids, bases, solvents (including methylene chloride) and radioactive materials (carbon 14). Labeling and handling procedures were generally adequate; however, operation of the hood was not properly checked. Filters in the hood should have been checked and static pressure measurements performed.

A complete inventory of equipment was not done. With the exception of the oven, equipment was generally adequate both in quantity and condition. The oven was potentially dangerous due to a

problem with the thermostat, and it was recommended that it be repaired or replaced. It was, however, clearly labeled, which minimized the risk of overheating.

A recommendation of the audit was to implement a data recording form for the carbon analyzer. This was done immediately after the audit. Another recommendation was to record date of preparation and preparer initials on all stock and standard solutions. This, too, was implemented. A standards logbook was not maintained. It was recommended that such logs be maintained, both to track chemical usage and to resolve problems related to contaminated chemicals or poor preparation practices.

Recommendations and Corrective Actions: Specific audit recommendations included the following: design and implement a data form for the carbon analyzer; implement a procedure for transcription error checks following computer data entry; improve and standardize logbook layout, including attachment of printer tapes (carbon analyzer) and identification of the analyst, document Oxymax calibrations, and make sure stocks and standards were properly labeled, including contents, date of preparation and identification. All of these specific recommendations were implemented with the exception of documenting Oxymax calibrations (the Oxymax was nonfunctional since the date of the audit). Application of QA/QC to microbiology is fairly new; most of the scientists in this laboratory were not familiar with the concepts of quality assurance. While much remained to be done, the understanding and application of QA within this laboratory improved over the duration of the project.

Audit: On-Site Audit of the Oil Chemistry Laboratory Operations at Valdez, Alaska
Date: August 9, 1990
Auditor: Mike Papp

Summary:

Facilities were small and cramped. Areas were generally well organized and well maintained to maximize efficiency of available space. One inadequacy was safe storage of chemicals--solvents were not properly stored.

A complete inventory of equipment and materials was not done. In general, equipment and materials were adequate, both in quantity and condition, for the needs of the project. The analysts record bench data could have been recorded neater.

Procedures, including application of QA/QC, were excellent. No problems were found and no recommendations were necessary.

Recommendations and Corrective Actions: No corrective actions were needed. Procedures appeared more than adequate to ensure high quality data.

One note--although the chemist wore a respirator while performing methylene chloride extracts, the extracts were not performed in the hood due to lack of space. Other individuals in the room could have been exposed.

SECTION 6
SNUG HARBOR FIELD RESULTS

VISUAL OBSERVATIONS

Test beaches at Snug Harbor were moderately contaminated. Visually, the cobble plots had a thin coating of dry, sticky, black oil covering rock surfaces and gravel areas under the cobble. Oil did not penetrate more than 8 to 10 cm below the gravel surface. In mixed sand and gravel plots, oil was well distributed over exposed surface areas and commonly found 20 to 30 cm below the surface. In many areas of the test plots small patches of thick oil and mousse could be found. This material was very viscous and mixed with extensive amounts of debris.

Approximately 8 to 10 days following oleophilic fertilizer application to the cobble beach plot, reductions in the amount of oil on rock surfaces were visually apparent. This was particularly evident from the air where the contrast with oiled areas surrounding the plot was dramatic, etching a clean rectangle on the beach surface (Figure 6.1). The contrast was also impressive at ground level; there was a precise demarkation between fertilizer-treated and untreated areas (Figure 6.2).

Close examination of this treated cobble plot showed that much of the oil on the surface of the rocks was gone. However, considerable amounts of oil were still present under rocks and in the mixed gravel below these rocks. The remaining oil was not dry and dull like the oil in other areas of the beach, but appeared softened and more liquid. It was also very sticky, with no tendency to come off the rocks. At the time of these observations, no oil slicks or oily materials were observed leaving the beach during tidal flushing.

There also appeared to be a reduction in the amount of oil in the mixed sand and gravel beach 2 to 3 weeks following oleophilic fertilizer application. However, visual differences between treated and untreated plots were not as dramatic as on the cobble beach. Some loss of subsurface oil in treated areas was also visually apparent. Reduction of oil contamination was particularly evident during the sampling process, as noticeably less oil remained on sampling equipment used on this beach plot. The amount of oil on all other plots at this time appeared unchanged since the initiation of the study. There were essentially no visual indications of oil removal on plots treated with slow- release fertilizer briquettes.

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Figure 6.1. Approximately 8 to 10 Days Following Application of INIPOL to the Cobble Beach Plot at Snug Harbor, Reductions in the Amount of Surface Oil (As Compared to Surrounding Untreated Oiled Areas) Was Evidenced by a Clean Rectangle on the Beach Surface.

Figure 6.2. At Ground Level, the Reduction in Oil on the INIPOL Treated Plot was also Strikingly Apparent.

Since several substances in INIPOL are known to act as surfactants or to otherwise change the consistency of oil on rock surfaces, the question arose as to whether INIPOL acted to alter the physical characteristics of the oil and could remove oil from rock surfaces without biodegradation. A study to evaluate the rock-washing characteristics of INIPOL under conditions that precluded biological activity showed that oleophilic fertilizer removed only 0.84% of the oil in a test where the fertilizer was added to ensure complete surface coverage. More than 30% of the oil was removed by some preparations sold specifically for this purpose. Approximately 45% of the oleophilic fertilizer, by weight, remained with the oiled rock in the testing regime used.

Thus, the oleophilic fertilizer did not wash oil off rocks at typical Prince William Sound water temperatures. Based on the results of this study, it is reasonable to expect that oil removal associated with oleophilic fertilizer applications was not the result of a chemical or physical process.

Over the next two to three weeks, the cleaned rectangle on the cobble beach remained clearly visible. Subsurface oil remained but was increasingly less apparent, and untreated reference plots appeared relatively unchanged. The oleophilic-treated mixed sand and gravel plot actually showed a greater loss of oil, appearing increasingly cleaner.

Six to eight weeks after fertilizer application the contrast between the treated and untreated areas on the cobble beach narrowed. This was due to reoiling from subsurface material concurrent with the slow removal of oil on the beach material surrounding the plot. However, it was evident that the total amount of oil on the treated plots had decreased substantially relative to untreated control plots. The corresponding mixed sand and gravel plot was also reoiled but to a lesser extent. Oil contamination was still observed at all other plots, but had generally decreased since the initiation of the study.

Toward the end of the summer season the area used for the bioremediation study became steadily cleaner, including most of the areas surrounding the test plots. This was attributed to several storms and more frequent rainfall. An untreated, heavily contaminated area to the south remained heavily contaminated throughout the summer by all visual criteria.

NUTRIENT CONCENTRATIONS

Figures 6.3a-e show the ammonia concentrations found in interstitial water for the treatment and reference plots. The initial background ammonia concentrations (T=0, Figure 6.3a) were low, and uniform throughout the plots. One to two days after application (T=1, Figure 6.3b) of the fertilizers, an increase in the ammonia concentrations was evident only in the plots treated with the oleophilic fertilizer. Concentrations within the zones, however, were highly variable. Based on the literature and laboratory nutrient release experiments described in Section 10, a pulse of ammonia was expected following application.

In contrast, ammonia concentrations in the plots treated with the slow-release briquettes remained at background levels. This is not unreasonable, because nutrient release studies with the briquettes showed nitrogen was released entirely as TKN, probably as urea. The absence of elevated NH_4 concentrations suggests that, on the beaches, hydrolysis of urea by microorganisms leads to immediate uptake of the resulting ammonia by bacteria or algae.

Eight to 10 days after application of the fertilizers (T=2, Figure 6.3c), ammonia concentrations were above background only in the sand and gravel plot treated with oleophilic fertilizer. Ammonia concentrations in plots treated with briquettes were comparable to the reference plot. At approximately 4 and 6 weeks after the fertilizer application (T=3, Figure 6.3d, and T=4, Figure 6.3e, respectively), no substantial difference in the ammonia concentrations was apparent between the treatment and the reference plots.

Figures 6.4a-c show nitrate/nitrite concentrations in interstitial water for the treatment and reference plots. One to 2 days following application (T=1, Figure 6.4a), notable concentrations of nitrate were found in samples taken from the briquette-treated beaches. Eight to 10 days after application (T=2, Figure 6.4b), sand and gravel beaches treated with oleophilic fertilizer showed substantially higher levels of nitrate/nitrite nutrients than the untreated control plots. Plots treated with water-soluble fertilizer showed only slightly elevated concentrations. One month after fertilizer application (T=3, Figure 6.4c), nitrate/nitrite levels in the treated plots were still higher than in the reference plots, particularly for the cobble beach treated with briquettes. Neither the INIPOL nor the briquettes contain nitrate or nitrite. Thus, it is possible that the presence of these nutrients resulted from ammonia conversion to nitrite by nitrification. If this was the case, it is unusual that it was confined only to the treated beaches.
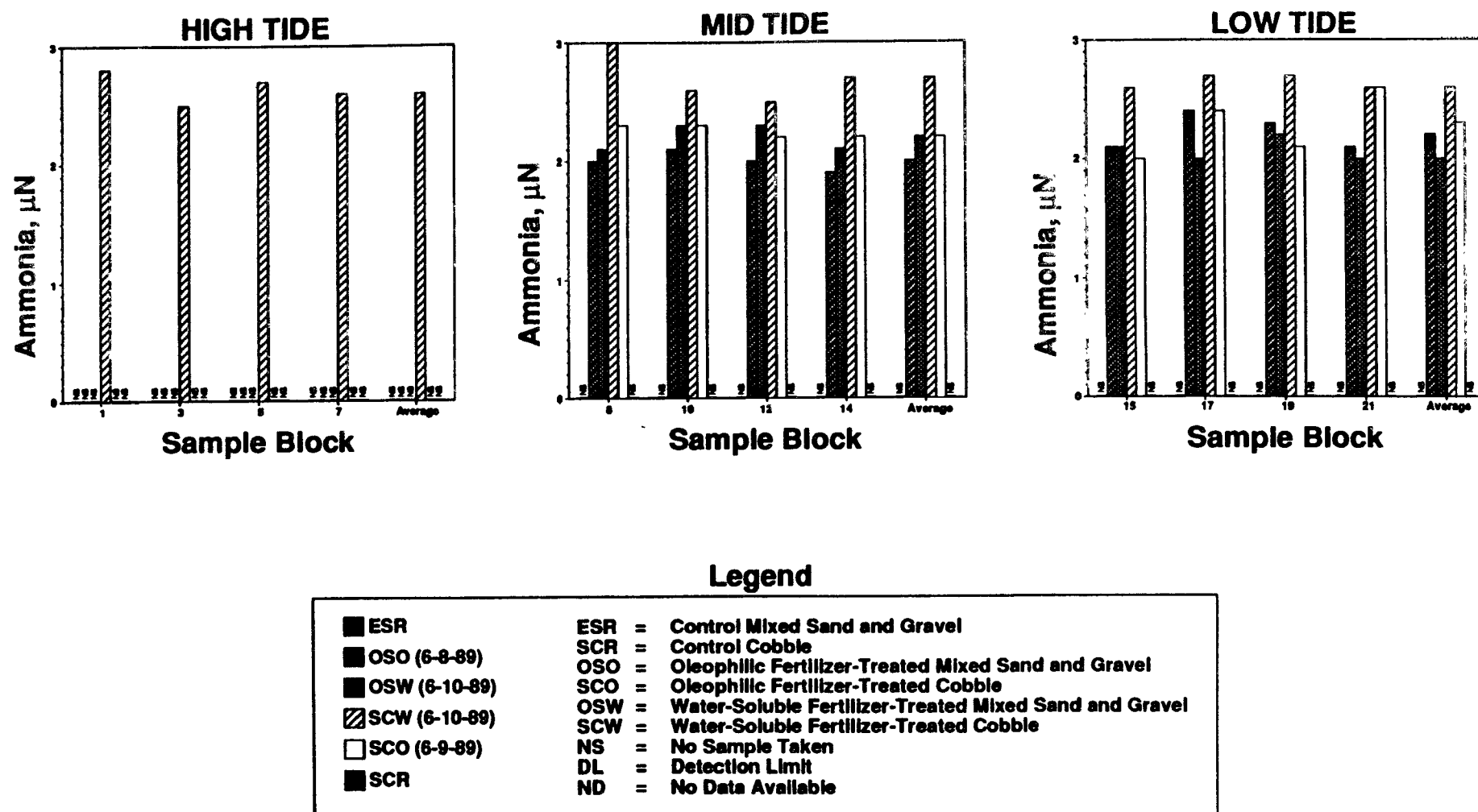
Figure 6.3a. Ammonia Concentrations In Interstitial Water Samples Prior To Fertilizer Application (T = 0).
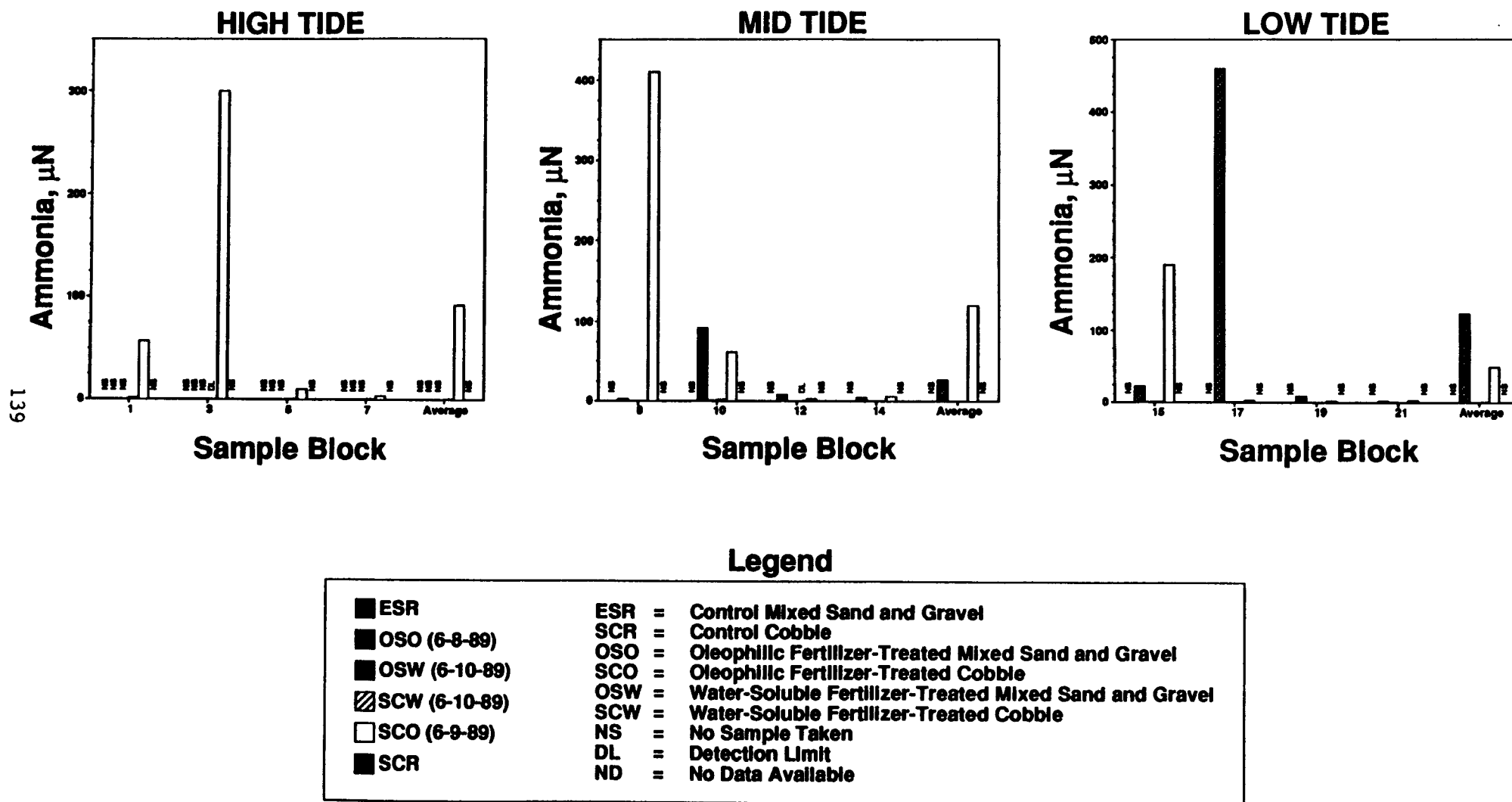
Figure 6.3b. Ammonia Concentrations In Interstitial Water Samples 1-2 Days Post Fertilizer Application (T = 1).

HIGH TIDE

Ammonia, μN

Sample Block

MID TIDE

Ammonia, μN

Sample Block

LOW TIDE

Ammonia, μN

Sample Block

Legend

■ ESR	ESR =	Control Mixed Sand and Gravel
■ OSO (6-8-89)	SCR =	Control Cobble
	OSO =	Oleophilic Fertilizer-Treated Mixed Sand and Gravel
■ OSW (6-10-89)	SCO =	Oleophilic Fertilizer-Treated Cobble
▨ SCW (6-10-89)	OSW =	Water-Soluble Fertilizer-Treated Mixed Sand and Gravel
	SCW =	Water-Soluble Fertilizer-Treated Cobble
☐ SCO (6-9-89)	NS =	No Sample Taken
	DL =	Detection Limit
■ SCR	ND =	No Data Available

Figure 6.3c.   **Ammonia Concentrations In Interstitial Water Samples 8-10 Days Post Fertilizer Application (T = 2).**

HIGH TIDE

Ammonia, μN

Sample Block

MID TIDE

Ammonia, μN

Sample Block

LOW TIDE

Ammonia, μN

Sample Block

Legend

■ ESR
■ OSO (6-8-89)
■ OSW (6-10-89)
▨ SCW (6-10-89)
☐ SCO (6-9-89)
■ SCR

ESR	=	Control Mixed Sand and Gravel
SCR	=	Control Cobble
OSO	=	Oleophilic Fertilizer-Treated Mixed Sand and Gravel
SCO	=	Oleophilic Fertilizer-Treated Cobble
OSW	=	Water-Soluble Fertilizer-Treated Mixed Sand and Gravel
SCW	=	Water-Soluble Fertilizer-Treated Cobble
NS	=	No Sample Taken
DL	=	Detection Limit
ND	=	No Data Available

Figure 6.3d. Ammonia Concentrations In Interstitial Water Samples 30 Days Post Fertilizer Application (T = 3).

HIGH TIDE

MID TIDE

LOW TIDE

Ammonia, μN

Sample Block

Legend

■ ESR	ESR	=	Control Mixed Sand and Gravel	
▓ OSO (6-8-89)	SCR	=	Control Cobble	
	OSO	=	Oleophilic Fertilizer-Treated Mixed Sand and Gravel	
■ OSW (6-10-89)	SCO	=	Oleophilic Fertilizer-Treated Cobble	
▨ SCW (6-10-89)	OSW	=	Water-Soluble Fertilizer-Treated Mixed Sand and Gravel	
	SCW	=	Water-Soluble Fertilizer-Treated Cobble	
☐ SCO (6-9-89)	NS	=	No Sample Taken	
	DL	=	Detection Limit	
■ SCR	ND	=	No Data Available	
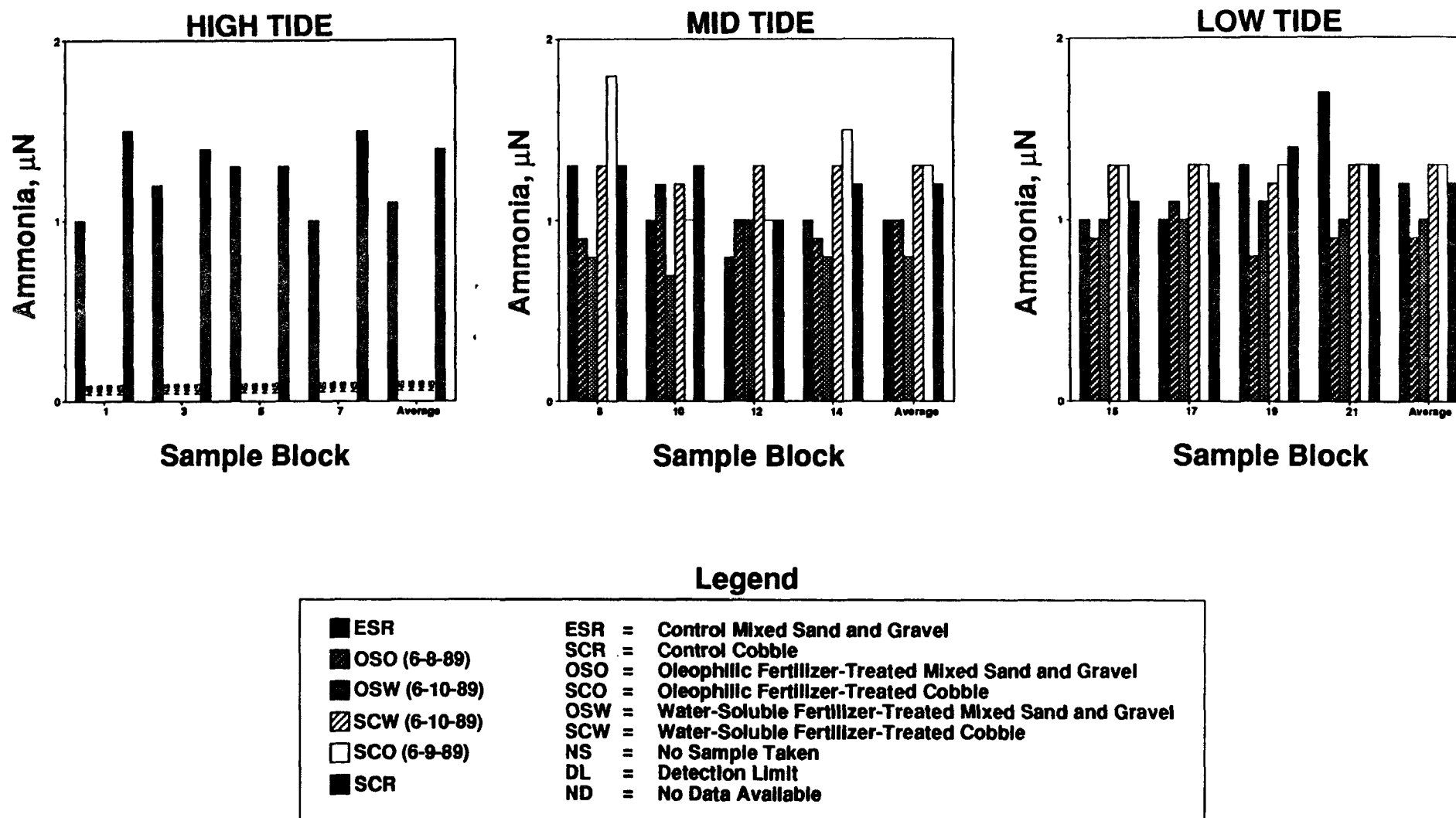
Figure 6.3e. Ammonia Concentrations In Interstitial Water Samples 6 Weeks Post Fertilizer Application (T = 4).
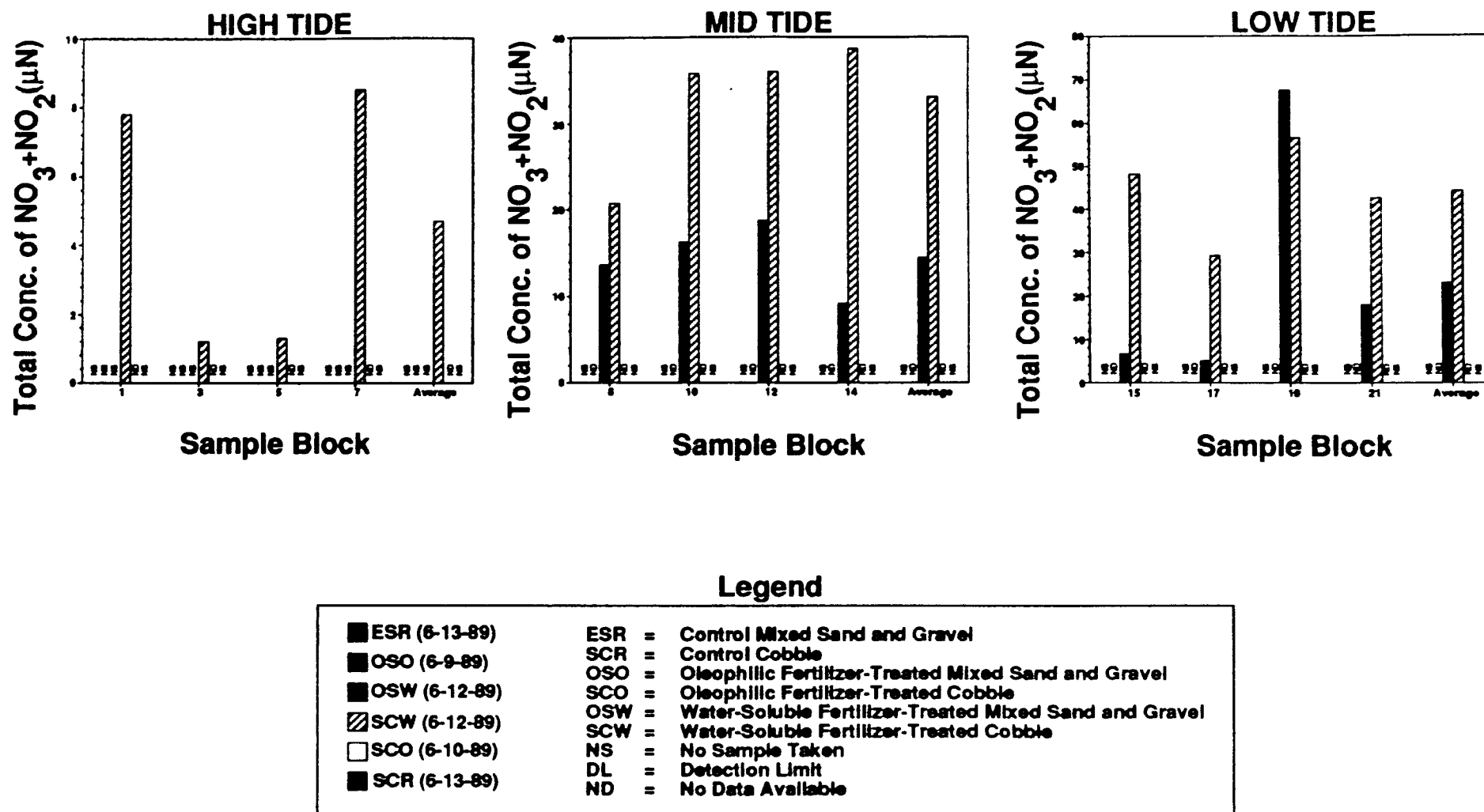
Figure 6.4a.   Nitrate / Nitrite Concentrations In Interstitial Water Samples 1-2 Days Post Fertilizer Application (T = 1).
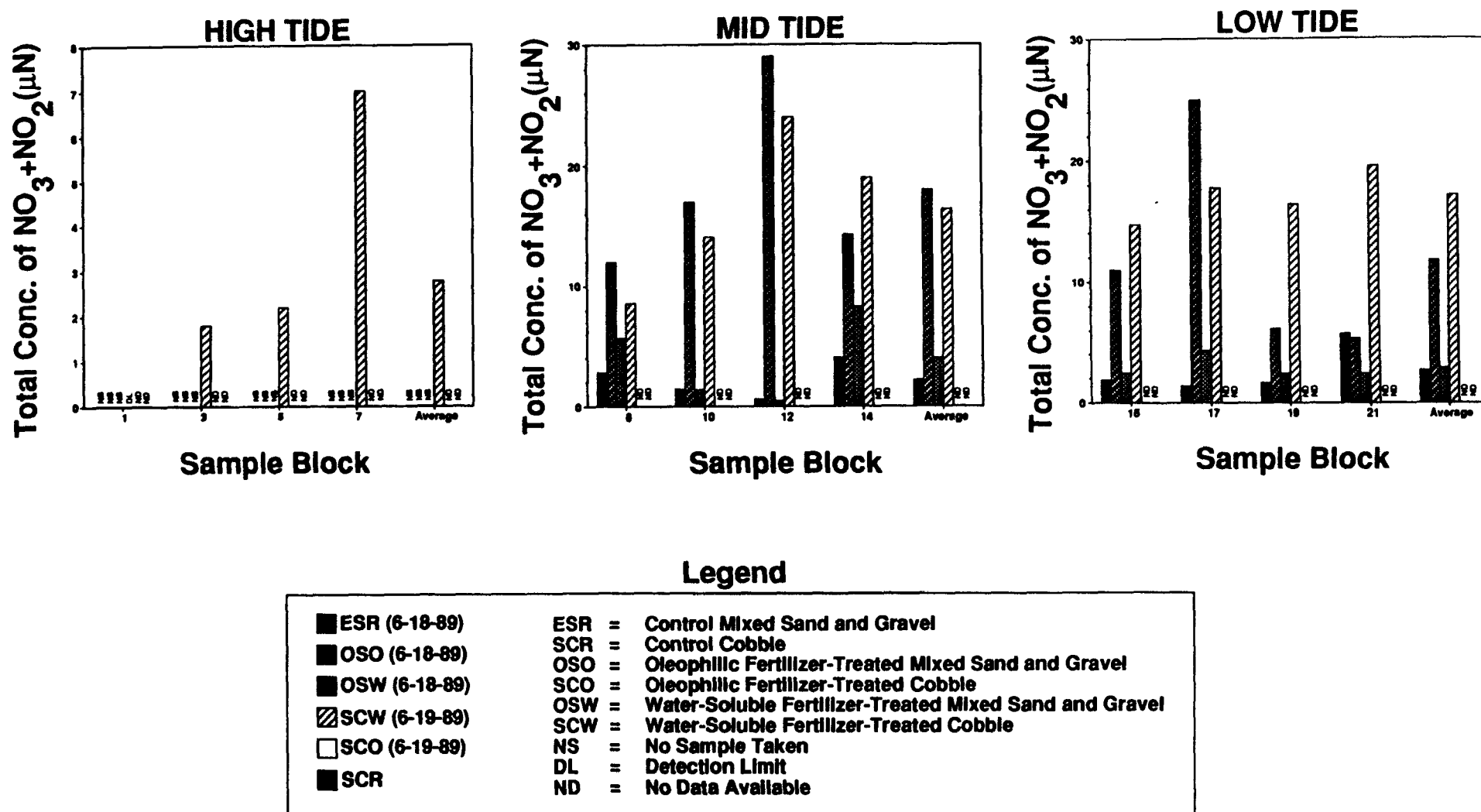
Figure 6.4b. Nitrate / Nitrite Concentrations In Interstitial Water Samples 8-10 Days Post Fertilizer Application (T = 2).

HIGH TIDE

MID TIDE

LOW TIDE

Sample Block

Sample Block

Sample Block

Legend

■ESR (7-7-89)	ESR	=	Control Mixed Sand and Gravel
■OSO (7-7-89)	SCR	=	Control Cobble
	OSO	=	Oleophilic Fertilizer-Treated Mixed Sand and Gravel
■OSW (7-7-89)	SCO	=	Oleophilic Fertilizer-Treated Cobble
▨SCW (7-7-89)	OSW	=	Water-Soluble Fertilizer-Treated Mixed Sand and Gravel
	SCW	=	Water-Soluble Fertilizer-Treated Cobble
☐SCO (7-7-89)	NS	=	No Sample Taken
	DL	=	Detection Limit
■SCR (7-7-89)	ND	=	No Data Available
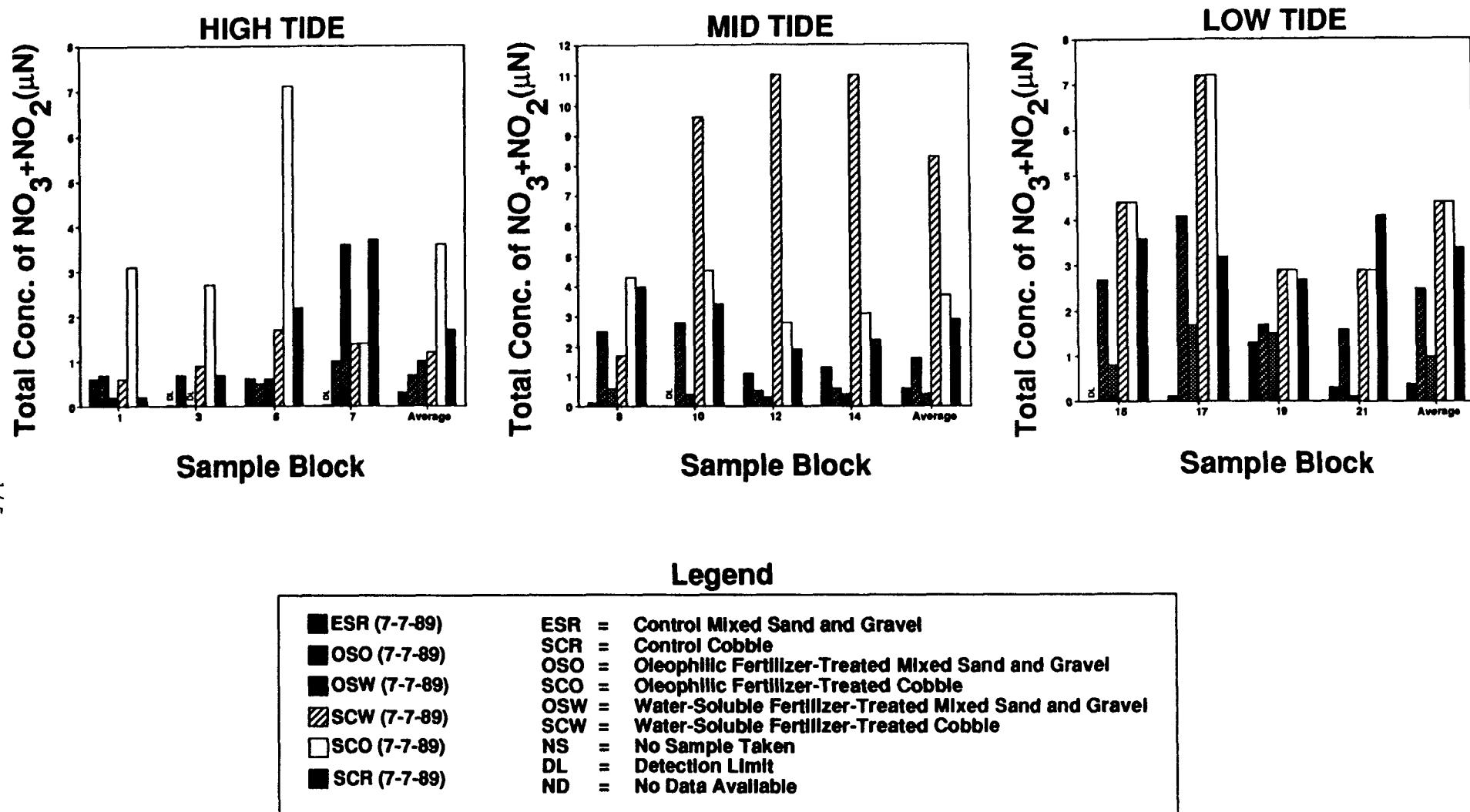
Figure 6.4c.   Nitrate / Nitrite Concentrations In Interstitial Water Samples
30 Days Post Fertilizer Application (T = 3).

Samples taken in July from streams near Eagle and Otter beaches showed measurable levels of inorganic nutrients. The stream south of Eagle beach had 5.2 μ nitrogen as nitrate. Stream samples taken adjacent to Otter beach contained an average of 4.8 μ nitrogen as nitrate. A sample of snow collected from a snow pile 300 yards southeast of Eagle Beach (a result of a winter avalanche) contained 2.8 μ of nitrogen as ammonia, 0.54 μ of phosphorus as phosphate, and 1.1 μ of nitrogen as nitrate. Although these concentrations were relatively low, they indicate that snow-melt and runoff may serve as important sources of nutrients for limited sections of the shoreline, particularly in the spring and early summer. Even though some of the test plots were located near the streams, nutrient concentrations in the plots were probably unaffected. The stream was an unlikely source of the nitrate found in the treated beaches, as no elevated nitrate/nitrite was detected in reference beaches having equal exposure to the freshwater. Also, no nitrate/nitrite was found at T=0 in any of the plots.

On June 19, the briquette bags were repositioned, and all bags were placed in the mid- and low-tide zones of the plots. The fertilizer was therefore submerged for longer time periods, enhancing nutrient transport in these zones. In general, this repositioning did not have a detectable impact on nutrient distribution on the beaches; nutrient concentrations in the zones showed no new trends. It was still apparent that minimal dispersion of the nutrients was occurring from the briquettes in areas of the shoreline not subjected to routine tidal washing. Precipitation during the month of June was probably insufficient to effectively transport nutrients released from the bags of briquettes located in the high-tide zone.

OIL CHEMISTRY

Oil Residue Weight

Cobble Plots (Seal Beach)

Figures 6.5 to 6.10 show changes (decay curves) in residue weights of oil on the cobble surface (referred to as cobble samples) and in the beach material below the cobble (referred to as mixed sand and gravel under cobble samples) for the three test plots on Seal Beach (untreated, treated with oleophilic fertilizer, and treated with fertilizer briquettes). The residue weights are normalized per kilogram weight of cobble or mixed sand and gravel. Given the relatively smooth surface of the cobblestones, it was assumed that a consistent relationship between rock surface area and rock weight existed. Variability in the residue weights was therefore a function of both the sampling of
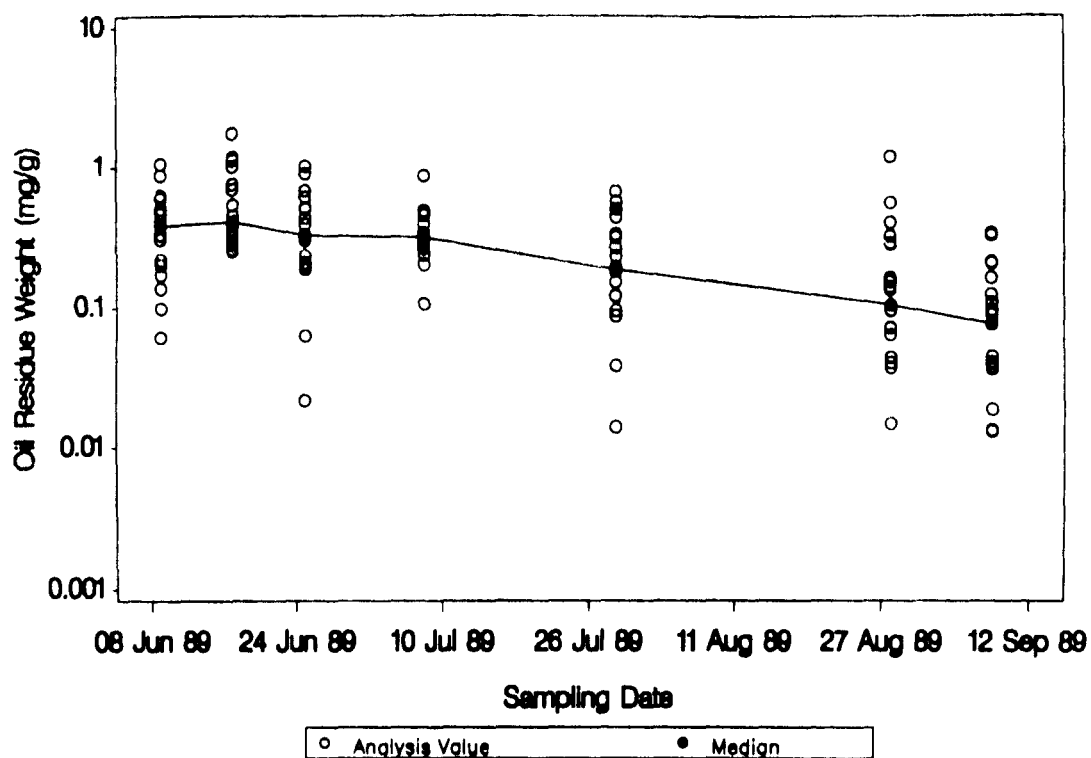
Figure 6.5.   Change In Oil Residue Weight Through Time for Seal Beach (Untreated Control) at Snug Harbor (Cobble Surface).
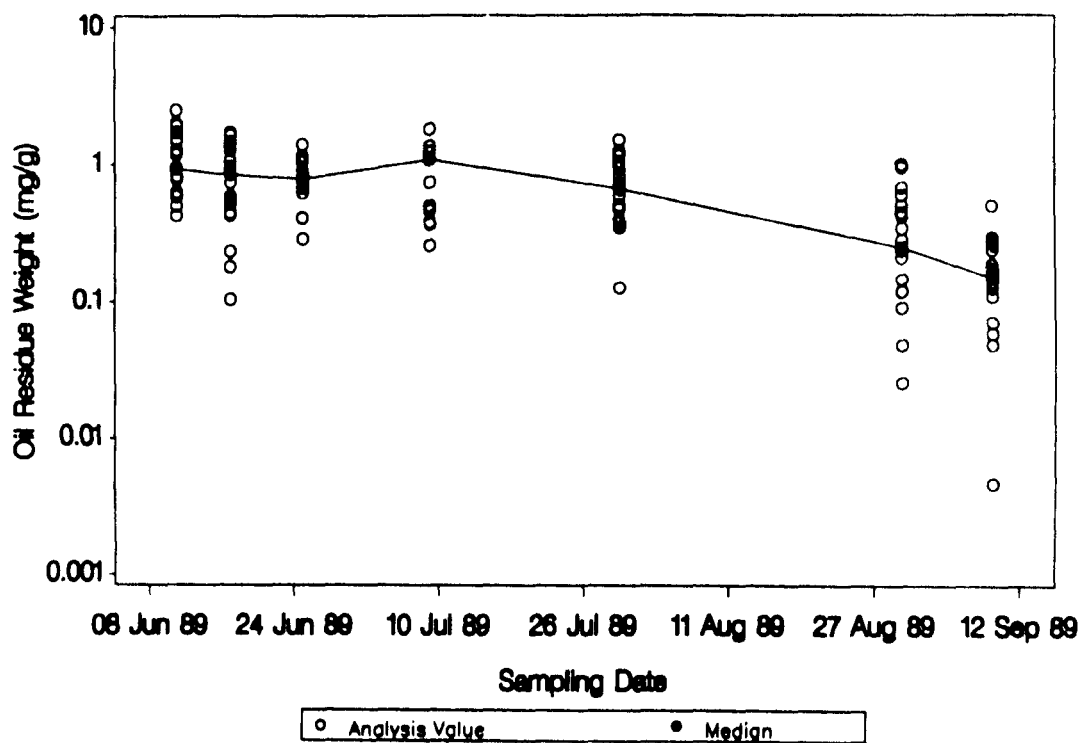
Figure 6.6.   Change In Oil Residue Weight Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Cobble Surface).
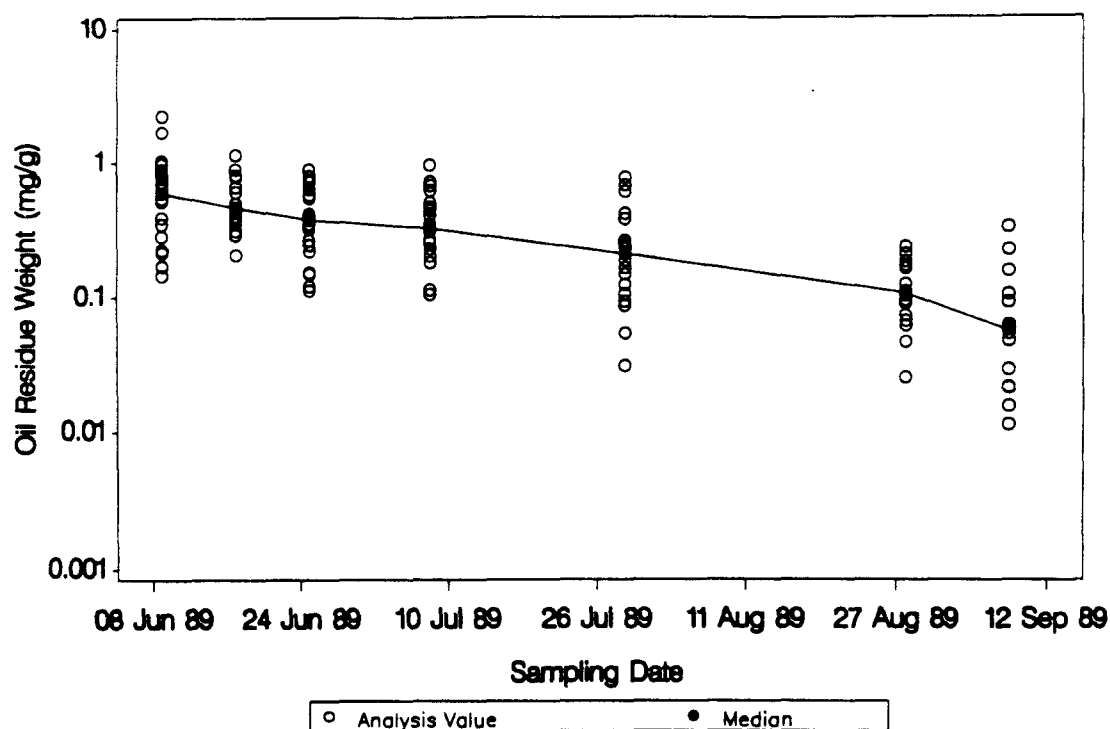
Figure 6.7. Change in Oil Residue Weight Through Time for Seal Beach (INIPOL) at Snug Harbor (Cobble Surface).
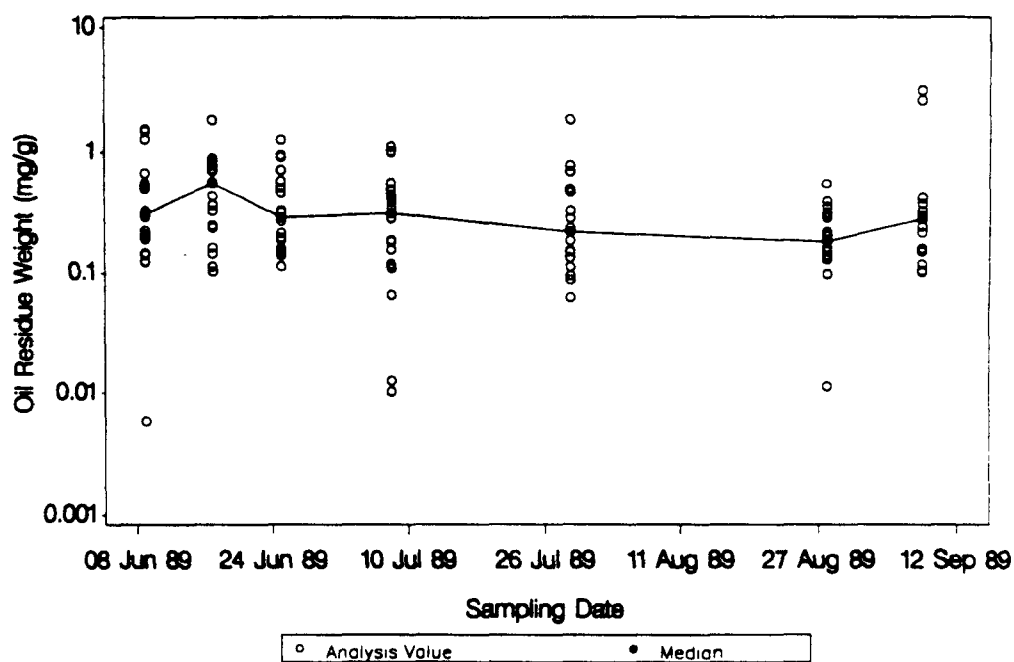
Figure 6.8. Change in Oil Residue Weight Through Time for Seal Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
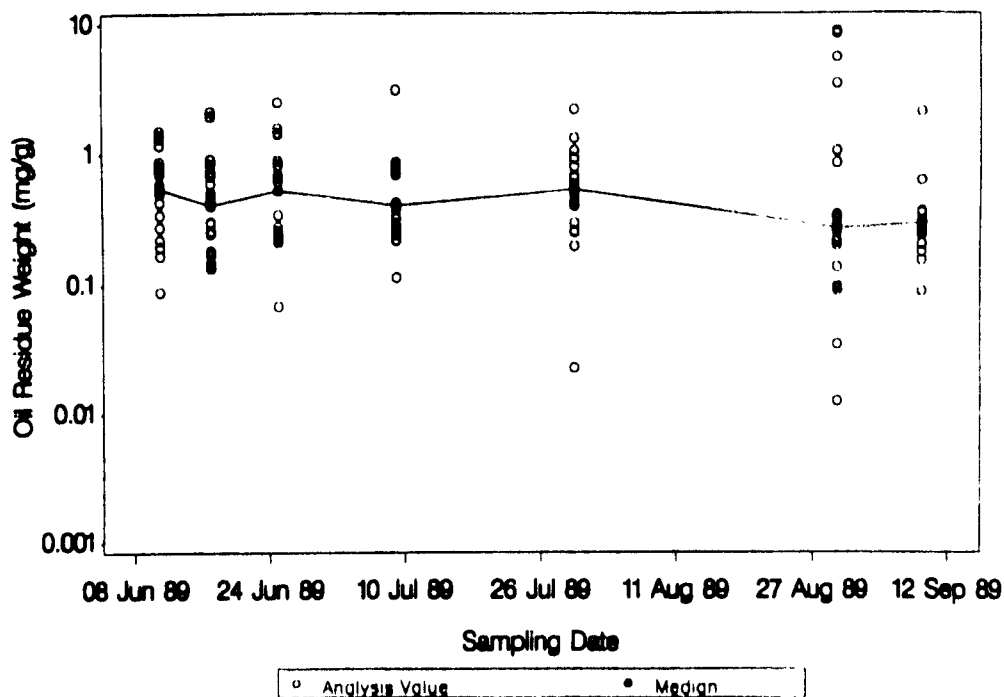
Figure 6.9. Change In Oil Residue Weight Through Time for Seal Beach (WOODACE Briquettes) for Snug Harbor (Mixed Sand and Gravel).
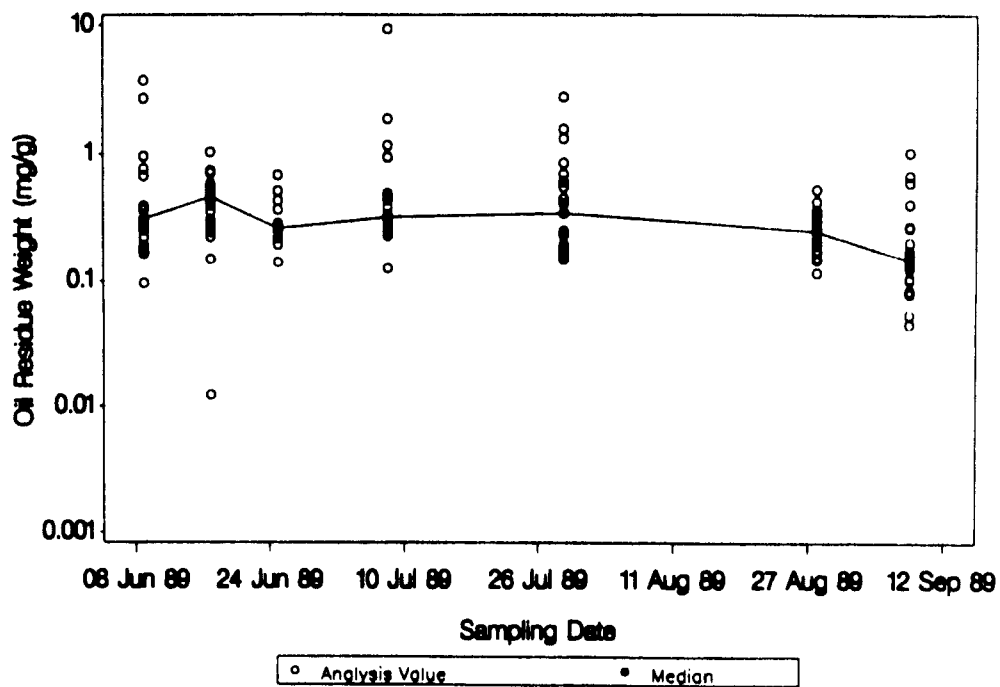
Figure 6.10. Change In Oil Residue Weight Through Time for Seal Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel).

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heterogenous beach material and the uneven distribution of oil on the beach. Because of this variability, the median values were used to develop the decay curves. Table 6.1 provides the actual median values and their percent change with each sampling date. Figure 6.11 graphically represents the percent change in the medians, and Table 6.2 compares the initial decay rate for each of the treatments.

Oil residue weight changes over the entire test period were essentially biphasic. On the untreated control beach, for example, there was relatively little change over the first 29 days of the test period, but this was then followed by a faster rate of decline. Comparing slopes of the two different phases (day 0 to day 29; day 29 to day 92) on this control plot showed that the initial rate is not significantly different from zero at the 95% confidence level (Table 6.2), but the latter slope was significantly different from zero. The reason for this change in rate between the July 8 and July 29 samplings is not known.

The decay curve for the briquette fertilizer-treated plot appeared to be biphasic as well, although an anonymously high median value on the July 8 sampling complicates the interpretation. However, the slopes of the two different phases (day 0 to day 29; day 29 to day 92) were statistically different from each other (Table 6.2), with the results generally mirroring those seen on the untreated control.

The decay curve for the INIPOL fertilizer-treated plot also appeared biphasic, but in a different sense; the initial decay was rapid and the latter decay was slower. During the first 29 days of the test period there was approximately a 45% decrease in the median oil residue weight on the INIPOL fertilizer-treated plot, but on the other plots there was essentially little change in the median values (Table 6.1). The greater weight loss on the INIPOL fertilizer-treated plot corresponds to the visual observations. Both phases showed slopes significantly different from zero. This response to the fertilizer application suggests that perhaps the presence of higher nutrient concentrations observed during the initial 2 to 3 weeks following application caused an enhancement of oil biodegradation rates, ultimately leading to greater initial loss of oil residues. However, other factors affecting the loss of oil residue cannot be ruled out (e.g., physical scouring by tide, wave action, chemical effects, etc.). If it was a nutrient effect, then it apparently was short-lived, as decay rates during the latter part of the test period were approximately the same as those seen on the untreated control plots.

TABLE 6.1. MEDIAN VALUES (MG/G) AND STATISTICAL COMPARISONS OF OIL RESIDUE WEIGHTS IN COBBLE SURFACE SAMPLES FROM DIFFERENT BEACH TREATMENTS AT SNUG HARBOR

Median Values (% of 6/9/89 Median)

Sampling Date	Day	Untreated Control	Briquettes	INIPOL
June 9	0	0.38	0.88	0.59
June 17	8	0.41 (108)	0.84 (96)	0.46 (78)
June 25	16	0.32 (85)	0.79 (90)	0.37 (63)
July 8	29	0.32 (85)	1.09 (124)	0.32 (55)
July 29	50	0.19 (49)	0.66 (75)	0.21 (35)
August 26	78	0.10 (27)	0.25 (28)	0.11 (18)
September 9	92	0.076 (20)	0.15 (17)	0.057 (10)

Mann-Whitney Test Results[a]

Sampling Date	Briquettes vs. INIPOL	Briquettes vs. Untreated Control	INIPOL vs. Untreated Control
June 9	B > I	B > C	I > C
June 17	B > I	Same	Same
June 25	B > I	B > C	Same
July 8	B > I	B > C	Same
July 29	B > I	B > C	Same
August 26	B > I	B > C	Same
September 9	B > I	B > C	Same

[a] 95 Percent Confidence Level
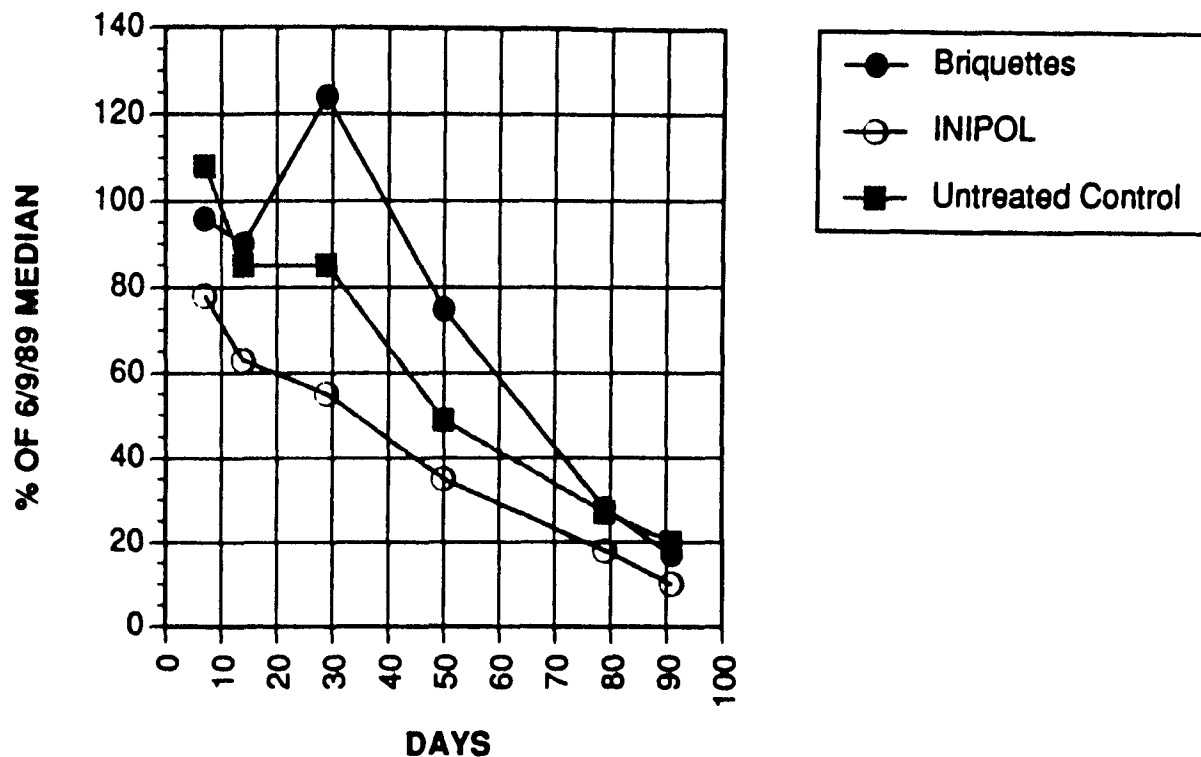
Figure 6.11. Change in the Median Residue Weight, Expressed as Percent of the 6/9/89 Median Over Time for the Briquette, INIPOL, and Untreated Control Beaches at Snug Harbor (Cobble Surface). All Variability is not Shown Because the Actual Data Points are not Presented.

TABLE 6.2. RATE ANALYSIS OF NATURAL LOG-TRANSFORMED OIL RESIDUE
WEIGHTS IN MG/G IN COBBLE SURFACE SAMPLES VERSUS TIME
(JULY 8, 1989 TO JULY 29, 1989 ONLY) FOR TEST BEACHES AT SNUG HARBOR

Beach	Slope (Std. Dev.)	Significance of Slope Greater than Zero			Half-Life, days	Time to Remove 90%, days
		N	T-value	p[a]		
Briquettes	-0.006 (0.007)	73	-0.82	0.42	122	404
INIPOL	-0.016 (0.007)	80	-2.4	0.02	44	146
Untreated Control	-0.006 (0.010)	65	-0.56	0.58	124	411

[a] Only the INIPOL rate is significantly different from zero at the 95 percent confidence level

It is unclear why the oil residue weight decay appeared to change suddenly around the beginning of July. If the response is related to nutrient availability, it is possible that the end of the algal spring bloom may have reduced the competition for available nutrients and provided nutrients for more oil degradation. However, the field nutrient data do not show much change in the background nutrient concentrations during this period of the test. It is also possible that the greatest decay in oil residue weight was due to the warm temperatures experienced in July.

Changes in oil residue weight through time for the mixed sand and gravel under cobble are shown in Figures 6.8 to 6.10. Decreases in residue weight decay were only apparent on the untreated control plot but the decreases was not significant (Table 6.3 and Figure 6.12). The oil residue weights on all plots were quite scattered, with median values randomly increasing or decreasing with time. In general, very low concentrations of oil were present; this may have been responsible for the scatter. Correspondingly, none of the decay rates on treated and untreated plots had slopes significantly different from zero (Table 6.4). Many samples on the untreated control plot had undetectable concentrations of oil, indicating that the oil on these plots was very heterogeneously distributed. It is also important to note

TABLE 6.3. MEDIAN VALUES (MG/G) AND STATISTICAL COMPARISONS OF OIL
RESIDUE WEIGHTS IN MIXED SAND AND GRAVEL SAMPLES UNDER COBBLE
FROM DIFFERENT BEACH TREATMENTS AT SNUG HARBOR

Median Values (% of 6/9/89 Median)

Sampling Date	Day	Untreated Control	Briquettes	INIPOL
June 9	0	0.32	0.57	0.31
June 17	8	0.50 (157)	0.40 (70)	0.47 (151)
June 25	16	0.31 (95)	0.57 (100)	0.26 (84)
July 8	29	0.35 (108)	0.40 (70)	0.31 (100)
July 29	50	0.22 (69)	0.56 (98)	0.35 (111)
August 26	78	0.20 (64)	0.27 (48)	0.25 (80)
September 9	92	0.28 (88)	0.29 (51)	0.14 (46)

Mann-Whitney Test Results[a]

Sampling Date	Briquettes vs. INIPOL	Briquettes vs. Untreated Control	INIPOL vs. Untreated Control
June 9	B > I	Same	Same
June 17	Same	Same	Same
June 25	B > I	B > C	Same
July 8	Same	Same	Same
July 29	B > I	B > C	Same
August 26	Same	Same	Same
September 9	B > I	Same	I < C

[a] 95 Percent Confidence Level
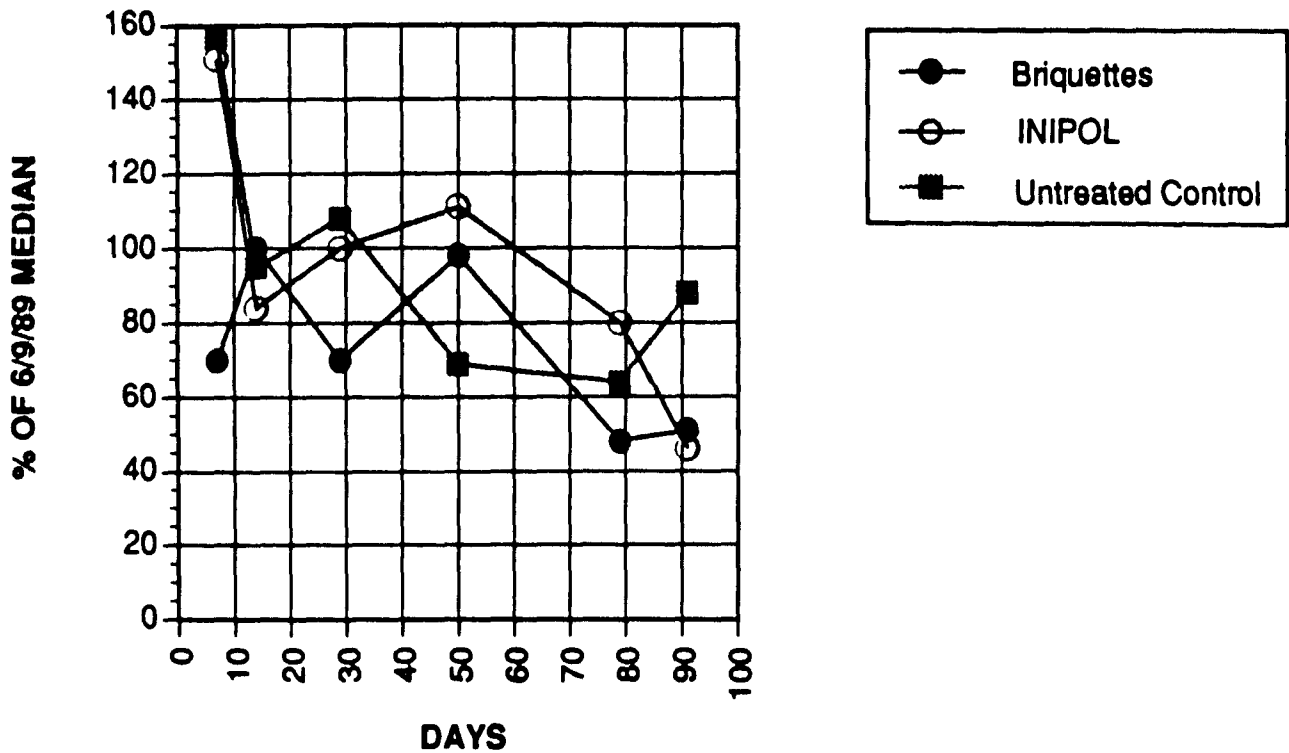
Figure 6.12. Change in the Median Residue Weight, Expressed as Percent of the 6/9/89 Median Over Time for the Briquette, INIPOL, and Untreated Control Beaches at Snug Harbor (Mixed Sand and Gravel Under Cobble). All Variability is not Shown Because the Actual Data Points are not Presented.

TABLE 6.4. RATE ANALYSIS OF NATURAL LOG-TRANSFORMED OIL RESIDUE
WEIGHTS IN MG/G IN MIXED SAND AND GRAVEL SAMPLES UNDER COBBLE
VERSUS TIME (JULY 8, 1989 TO JULY 29, 1989 ONLY)
FOR TEST BEACHES AT SNUG HARBOR

Beach	Slope (Std. Dev.)	Significance of Slope Greater than Zero		
		N	T-value	p[a]
Briquettes	0.008 (0.010)	81	0.85	0.40
INIPOL	-0.0008 (0.008)	78	-0.11	0.91
Untreated Control	-0.007 (0.009)	77	-0.84	0.40

[a] None of the rates are statistically different from zero at the 95 percent confidence level

that the INIPOL application to the cobble surface did not displace the oil and cause it to collect in the mixed sand and gravel below; that is, the apparent large increase in oil residue weight in the mixed sand and gravel on the INIPOL-treated plot at the first sampling after fertilizer application was also observed on the untreated control plot.

Mixed Sand and Gravel Plots (Eagle and Otter Beaches)

The decay in oil residue weight on the beaches consisting only of mixed sand and gravel is shown in Figures 6.13 to 6.15 and Table 6.5. Figure 6.16 showed that the percent change in the median oil residue weights with respect to the Day 0 sampling. There appears to be a slow steady decay on both the untreated control and INIPOL fertilizer-treated plots. However, the slopes of these decay curves (Table 6.6) were not statistically different from a slope of zero. Unfortunately, the beaches selected for the test were considerably different in terms of oil contamination, with the untreated control plot containing almost five times more oil (Table 6.5). This difference may have obscured any significant differences between the untreated control and treated plots. In addition, there was a very large drop in oil concentration on both the untreated control plot and the INIPOL fertilizer-treated plot between the t = 0 and t = 8 day samplings. The cause of these initial decreases is not clear.
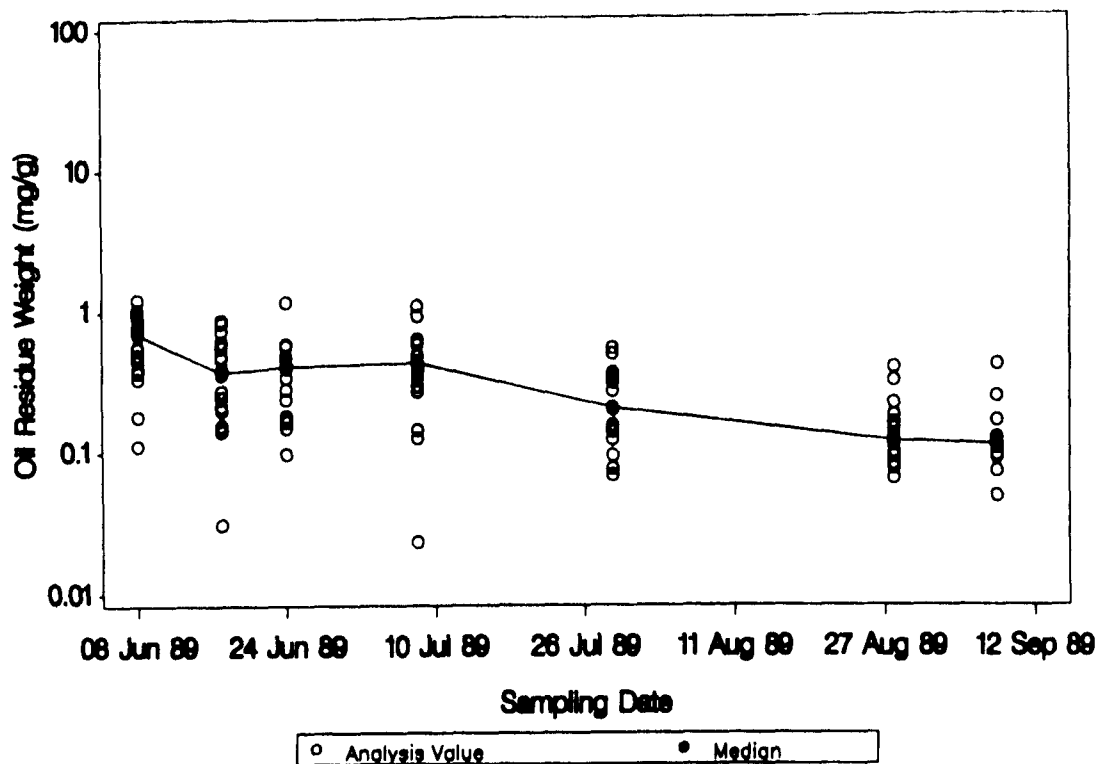
Figure 6.13. Change in Oil Residue Weight Through Time for Otter Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel).
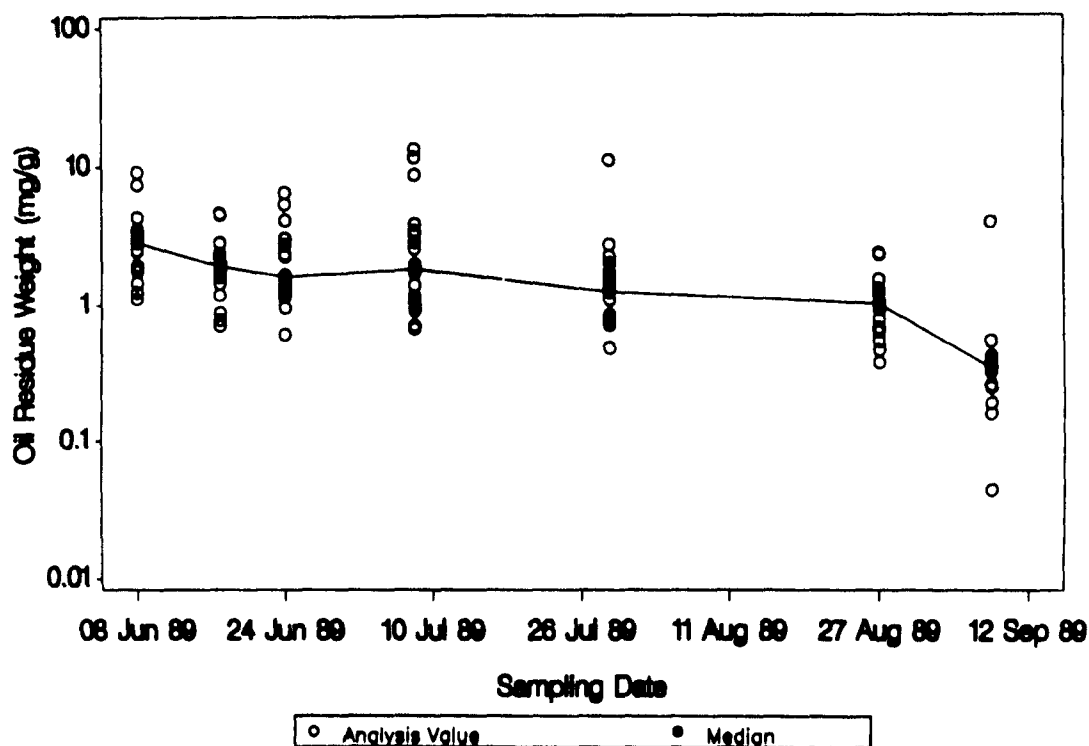
Figure 6.14. Change in Oil Residue Weight Through Time for Eagle Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel).
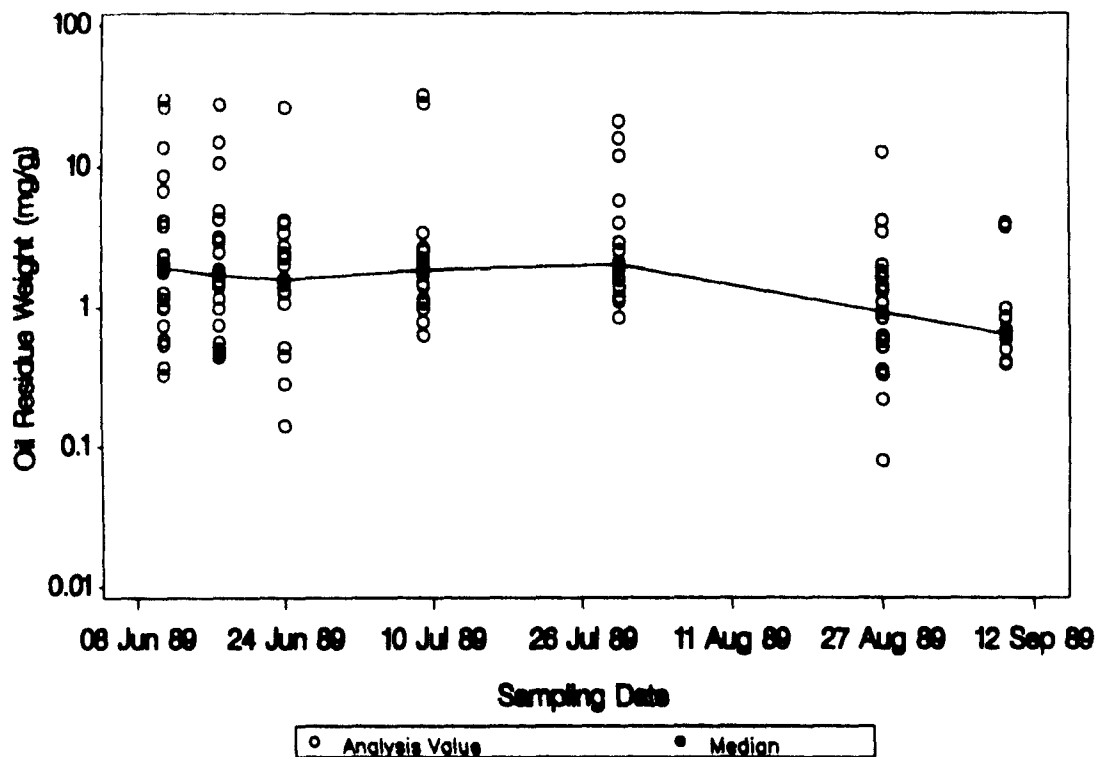
Figure 6.15. Change In Oil Residue Weight Through Time for Otter Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel).
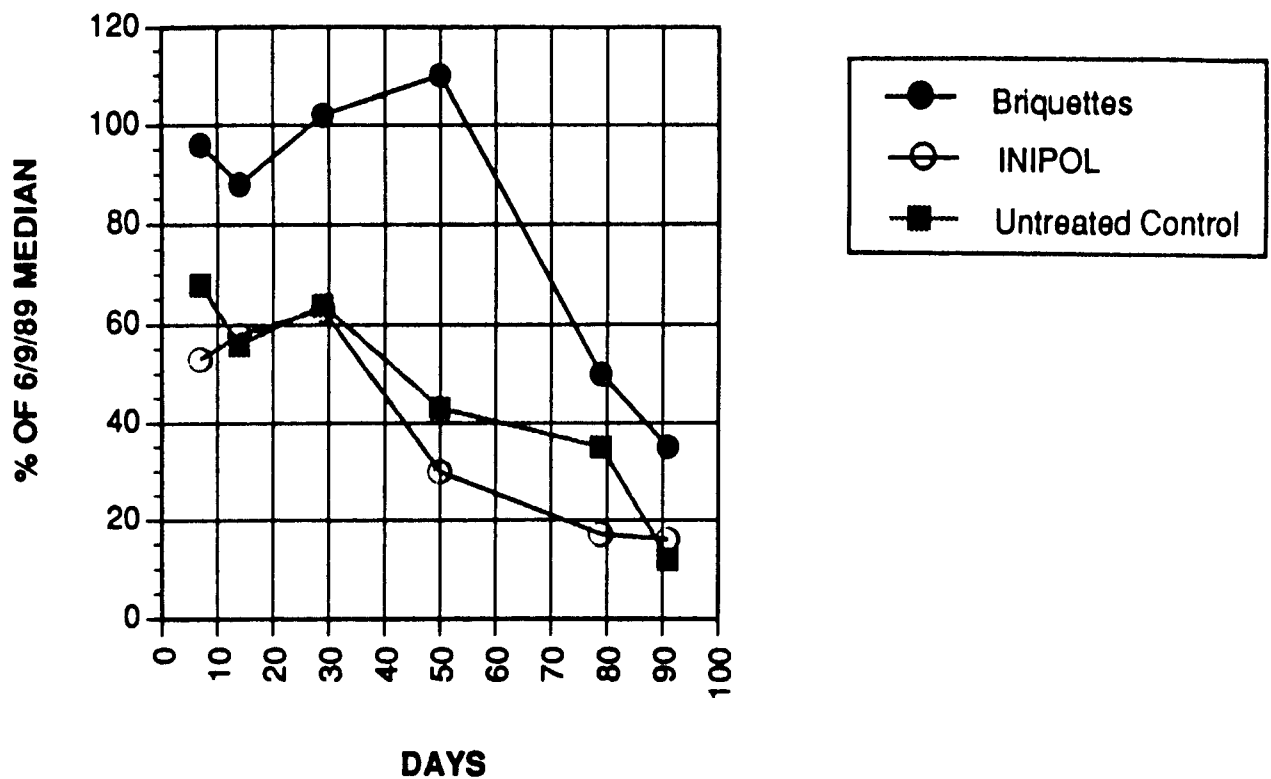
Figure 6.16. Change In the Median Residue Weight, Expressed as Percent of the 6/9/89 Median Over Time for the Briquette, INIPOL, and Untreated Control Beaches at Snug Harbor (Mixed Sand and Gravel Only). All Variability Is not Shown Because the Actual Data Points are not Presented.

TABLE 6.5. MEDIAN VALUES (MG/G) AND STATISTICAL COMPARISONS OF OIL
RESIDUE WEIGHTS IN MIXED SAND AND GRAVEL ONLY SAMPLES
FROM DIFFERENT BEACH TREATMENTS AT SNUG HARBOR

Median Values (% of 6/9/89 Median)

Sampling Date	Day	Untreated Control	Briquettes	INIPOL
June 9	0	2.85	1.80	0.71
June 17	8	1.92 (68)	1.73 (96)	0.37 (53)
June 24	16	1.60 (56)	1.58 (88)	0.41 (58)
July 8	29	1.82 (64)	1.83 (102)	0.45 (63)
July 29	50	1.23 (43)	1.98 (110)	0.21 (30)
August 26	78	0.99 (35)	0.90 (50)	0.12 (17)
September 9	92	0.34 (12)	0.63 (35)	0.11 (16)

Mann-Whitney Test Results[a]

Sampling Date	Briquettes vs. INIPOL	Briquettes vs. Untreated Control	INIPOL vs. Untreated Control
June 9	B > I	Same	I < C
June 17	B > I	Same	I < C
June 24	B > I	Same	I < C
July 8	B > I	Same	I < C
July 29	B > I	B > C	I < C
August 26	B > I	Same	I < C
September 9	B > I	B > C	I < C

[a] 95 Percent Confidence Level

TABLE 6.6. RATE ANALYSIS OF NATURAL LOG-TRANSFORMED OIL RESIDUE
WEIGHTS IN MG/G IN MIXED SAND AND GRAVEL ONLY SAMPLES
VERSUS TIME (JULY 8, 1989 TO JULY 29, 1989 ONLY)
FOR TEST BEACHES AT SNUG HARBOR

| Beach | Slope (Std. Dev.) | Significance of Slope Greater than Zero | | |
		N	T-value	p[a]
Briquettes	-0.0008 (0.012)	81	-0.07	0.94
INIPOL	-0.008 (0.007)	82	-1.25	0.21
Untreated Control	-0.007 (0.007)	80	-0.97	0.33

[a] None of the rates are statistically significant from zero at the 95 percent confidence level

Also, oil residue loss on the briquette fertilizer-treated plot did not appear to change substantially until the August 26 sampling when a very precipitous decrease (>50% change in the median in the residue weight) occurred. Again, no explanation for this decrease is available.

Oil Composition

Cobble Plots (Seal Beach)

a) Cobble Surface Samples

Changes through time in the concentration of selected normal alkanes (nC18, nC22, nC27), the sum of normal alkanes (nC18 to nC27), the branched alkanes pristane and phytane, and the nC18/phytane ratios in cobble surface samples taken from the treated and untreated control plots, are shown in Figures 6.17 to 6.37. All values of hydrocarbon concentration were normalized to the weight of oil in the extracted sample. The normal alkanes nC18 to nC27 were chosen because correlation analysis showed that these hydrocarbons tracked each other consistently. In all cases values below detection limits were treated as zeros.

For comparative purposes, Tables 6.7 and 6.8 summarize the percent change in the medians of individual hydrocarbons and the number of samples showing a hydrocarbon concentration of zero (below detection limits) with each sampling period, respectively. Figure 6.38 provides a graphical representation of the percent change in the medians for several hydrocarbons.

Several general trends can be identified from this data. For the cobble surface samples from all beach plots, there appeared to be considerable differences between the treated plots and the untreated control plot during the first 8 to 16 days of the test period. In most cases, median concentrations of all the individual normal alkanes decreased by at least 40%, and in some cases considerably more during this initial period. This was particularly true on the INIPOL fertilizer-treated plot (Figures 6.29 to 6.34 and 6.38; Table 6.7) where in some cases decreases were almost an order of magnitude. Similar decreases were not seen on the untreated control plot (Figures 6.17 to 6.22 and 6.38; Table 6.7). Statistical analysis using the Mann-Whitney test (Table 6.7) showed no statistical difference in alkane concentration between the first three sampling dates on the untreated control plot; in other words, a lag had occurred in the hydrocarbon decay. This was not the case on the fertilizer-treated plots; median alkane concentrations on the 8-day sampling were significantly less than the initial median concentrations.

Changes following these initial responses, however, were considerably more complex. The alkane concentrations on the untreated control plot subsequent to the 16-day sampling (June 25; Figures 6.17 to 6.22 and 6.38) generally decayed steadily and slowly, with a somewhat faster decay for lower molecular weight alkanes. Alkane concentrations on the briquette fertilizer-treated plots (Figures 6.23 to 6.28 and 6.38) appeared to change relatively little subsequent to the 16-day sampling (following the July 29 sampling, alkane decay on all plots dramatically accelerated for reasons that are not immediately obvious). If decay rates for the summed alkanes covering the initial 50 days of the test are analyzed statistically (Tables 6.9 and 6.10), the slopes are statistically different from zero for both the untreated control and the briquette fertilizer-treated plots. However, the influence of the initial drop on the briquette fertilizer-treated plot was significant. That is, if the t=0 sampling is not considered then only the slope on the untreated control plot was significantly different from zero. Thus, it appears that the application of fertilizer briquettes had an initial effect on the oil composition with respect to normal alkanes, but over time less compositional change occurred than on the untreated control. This could have been related to the considerably higher oil loading on the cobble surface of the briquette fertilizer-treated plot, requiring more time to see significant changes in oil composition (see below).
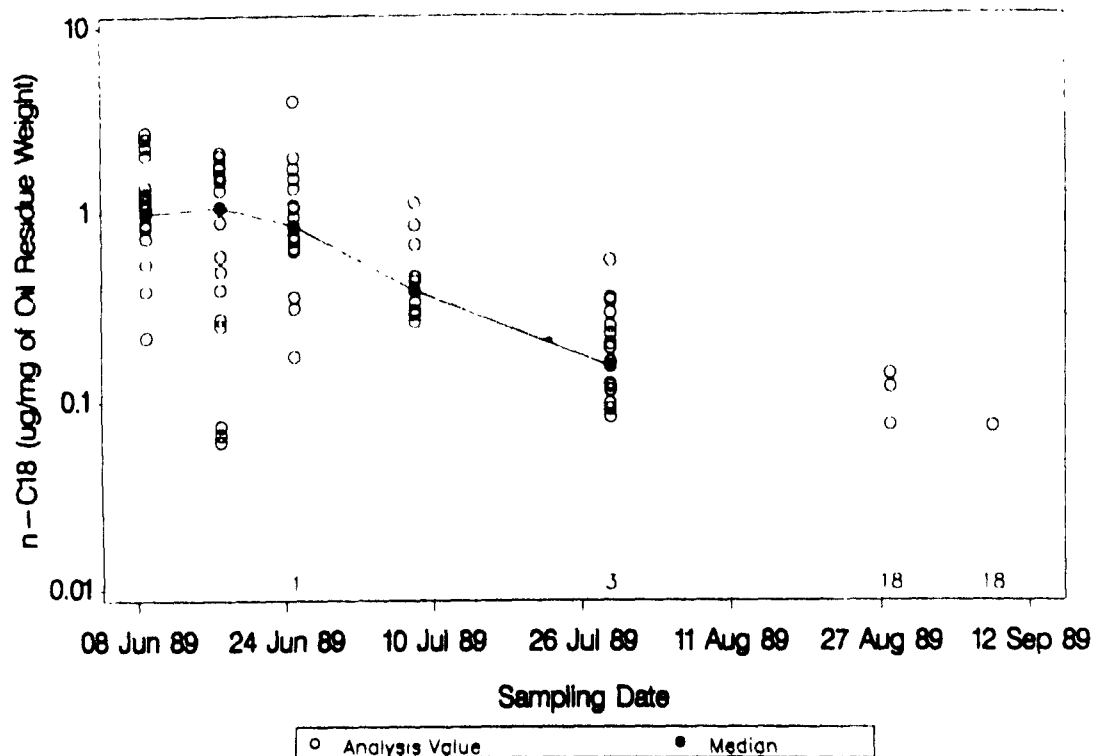
Figure 6.17. Change in nC18 Concentration Through Time for Seal Beach (Untreated Control) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
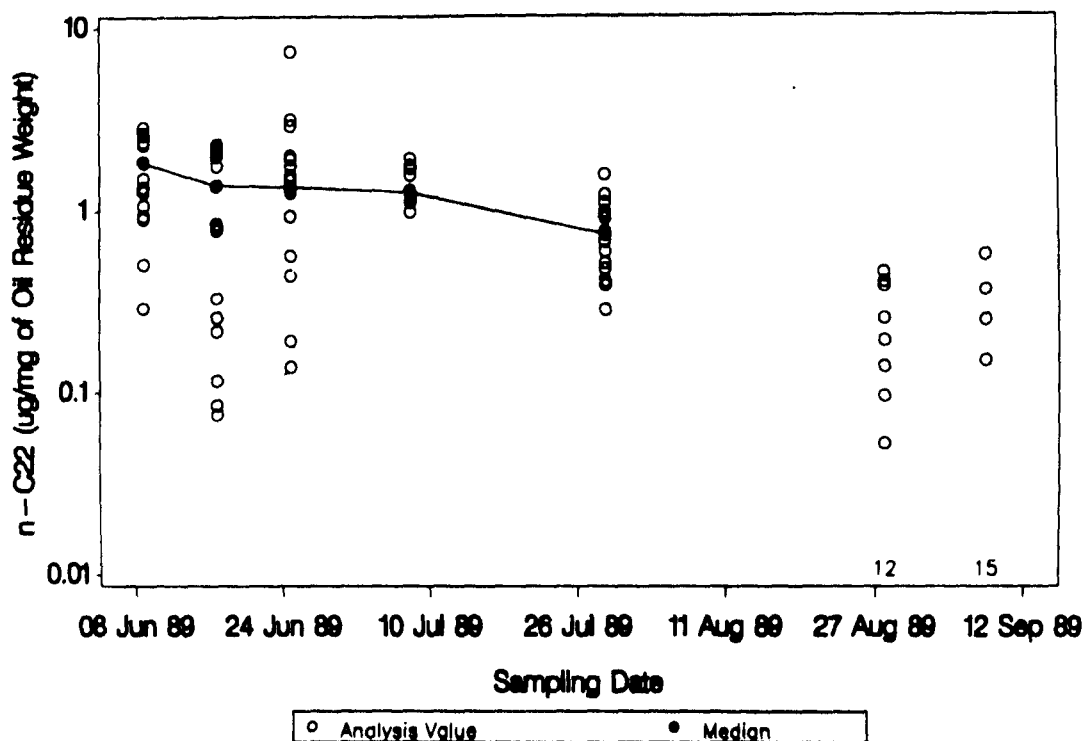
Figure 6.18. Change in nC22 Concentration Through Time for Seal Beach (Untreated Control) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

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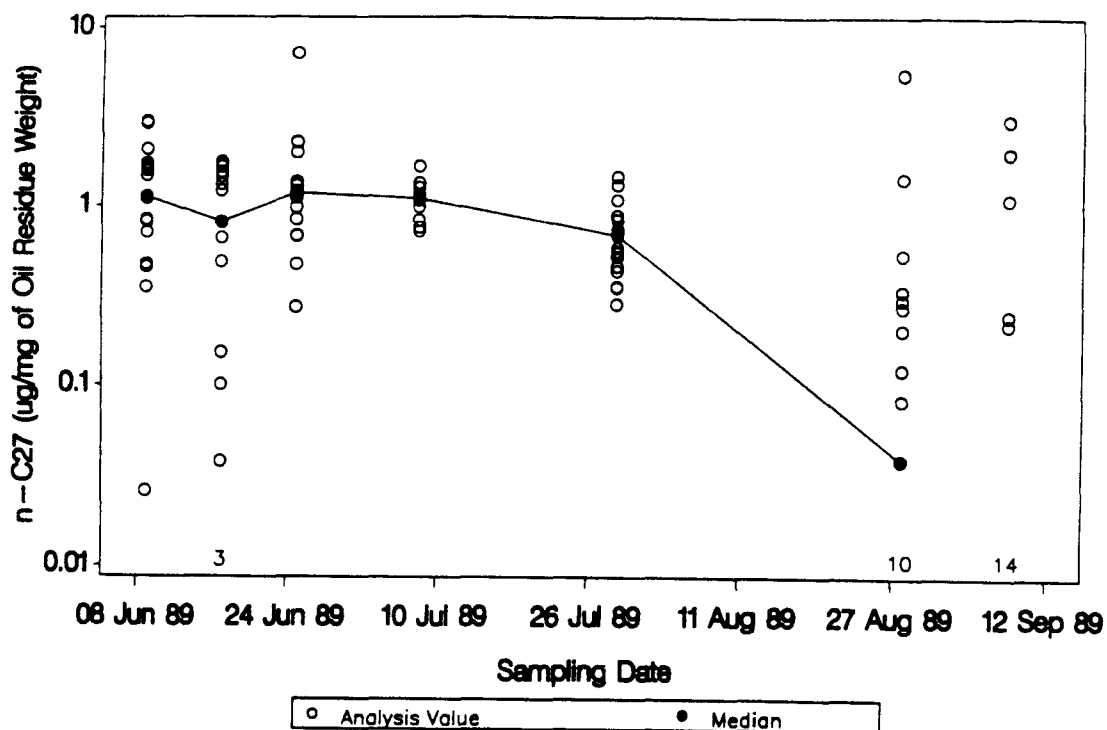
Figure 6.19. Change in nC27 Concentration Through Time for Seal Beach (Untreated Control) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
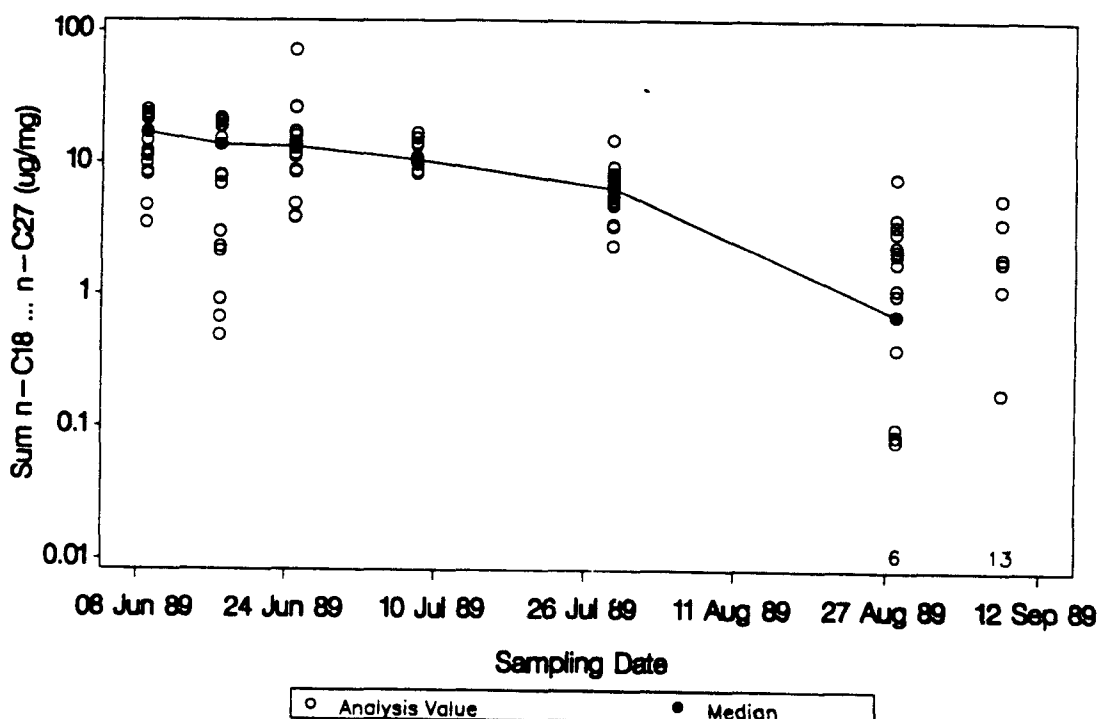
Figure 6.20. Change in Sum of Alkane Concentration nC18 to nC27 Through Time for Seal Beach (Untreated Control) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

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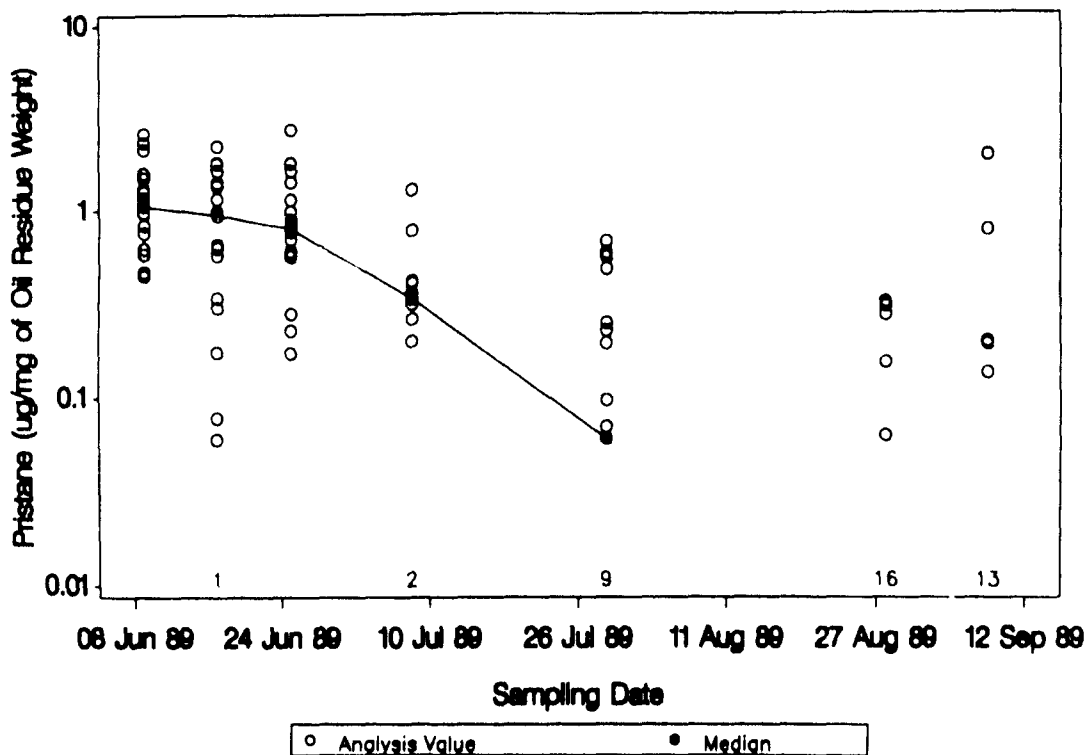
**Figure 6.21.** Change in Pristane Concentration Through Time for Seal Beach (Untreated Control) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
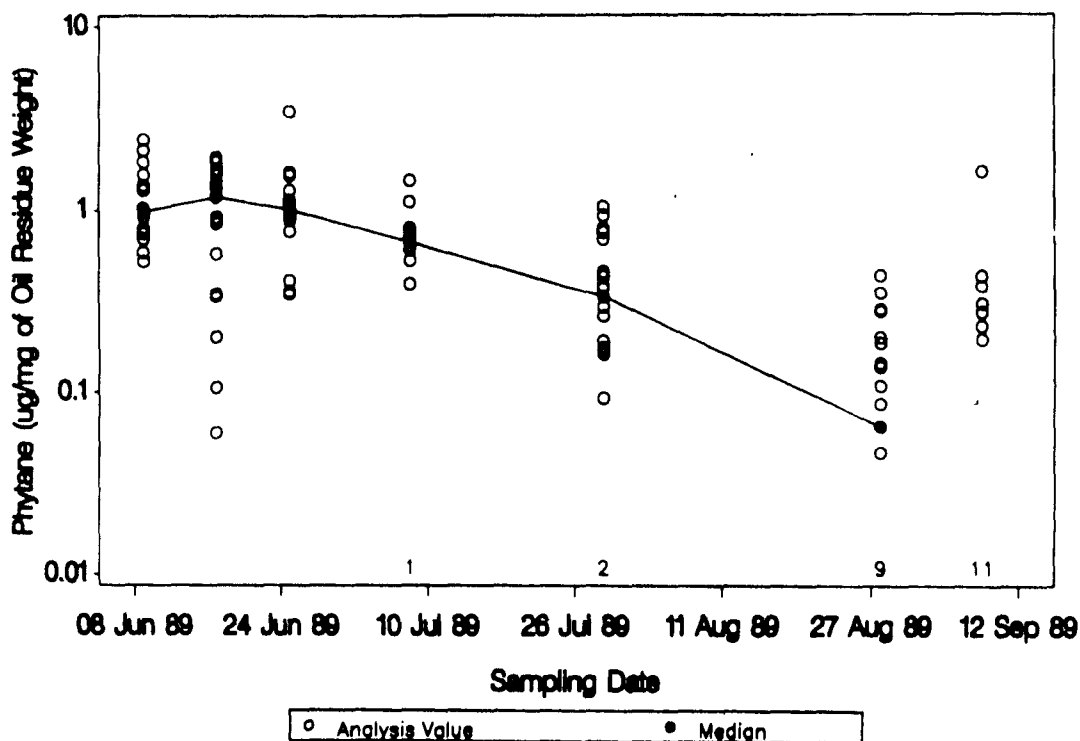
Figure 6.22. Change in Phytane Concentration Through Time for Seal Beach (Untreated Control) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
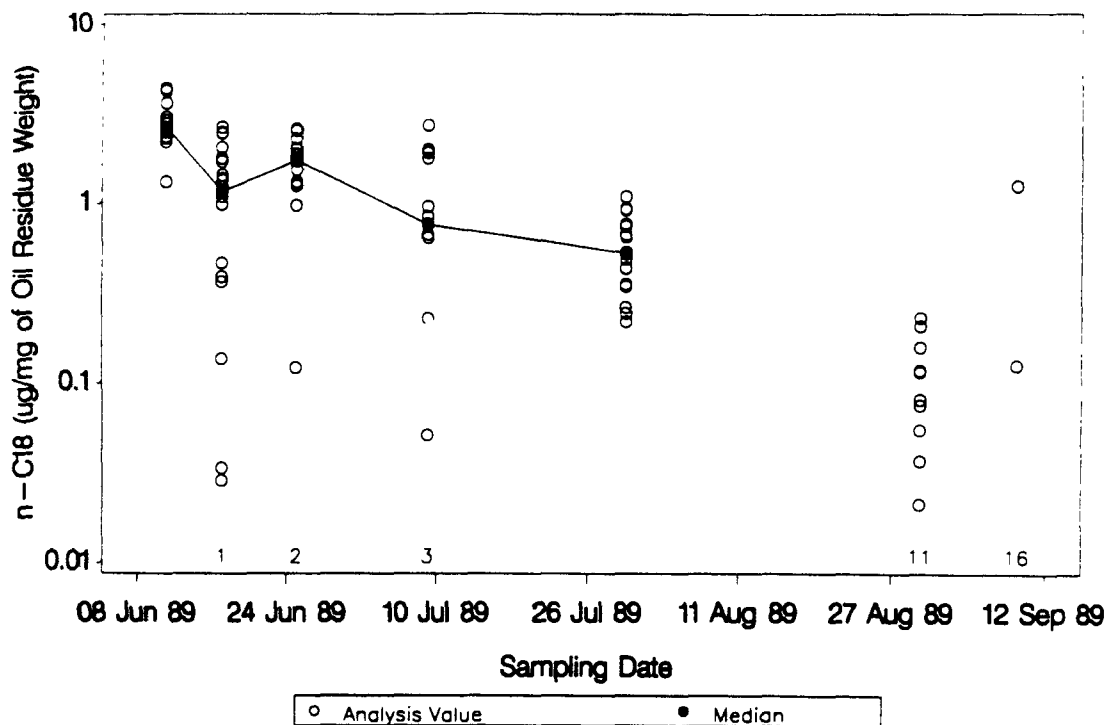
Figure 6.23. Change in nC18 Concentration Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
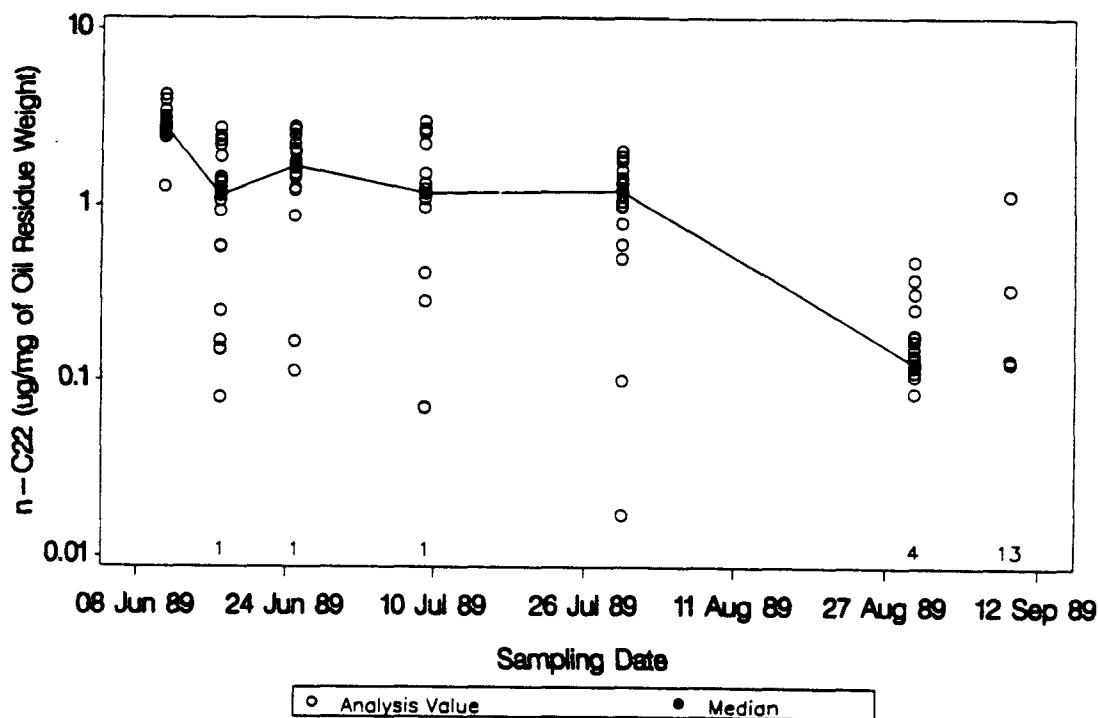
Figure 6.24. Change in nC22 Concentration Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

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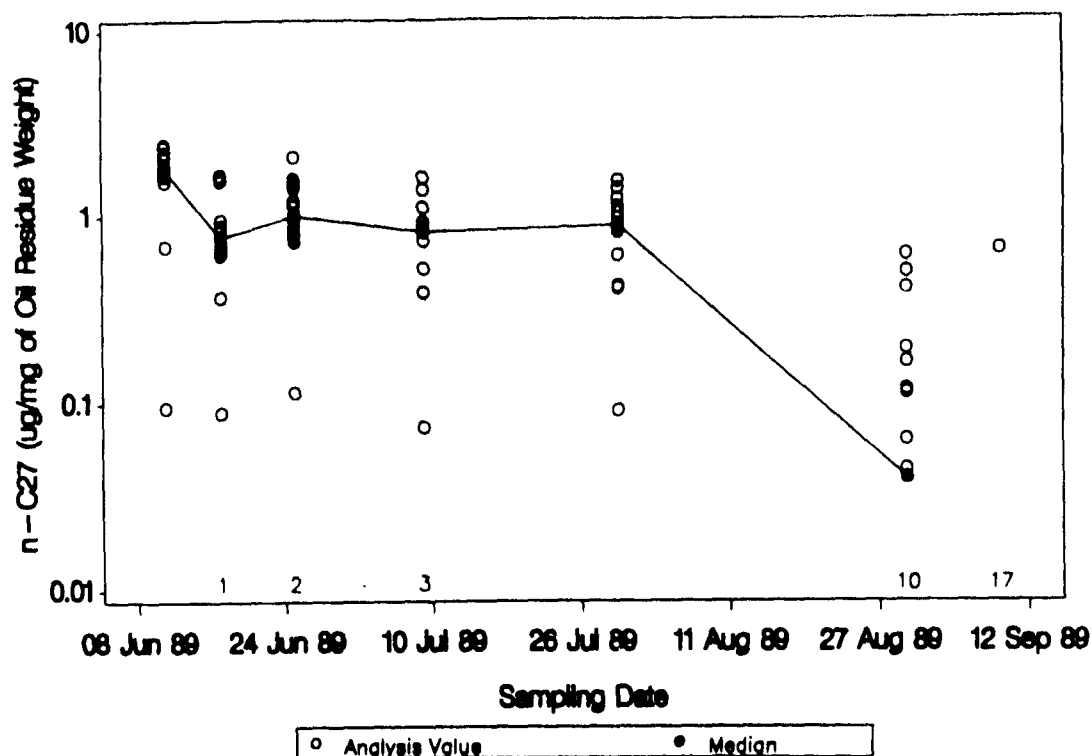
Figure 6.25. Change in nC27 Concentration Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
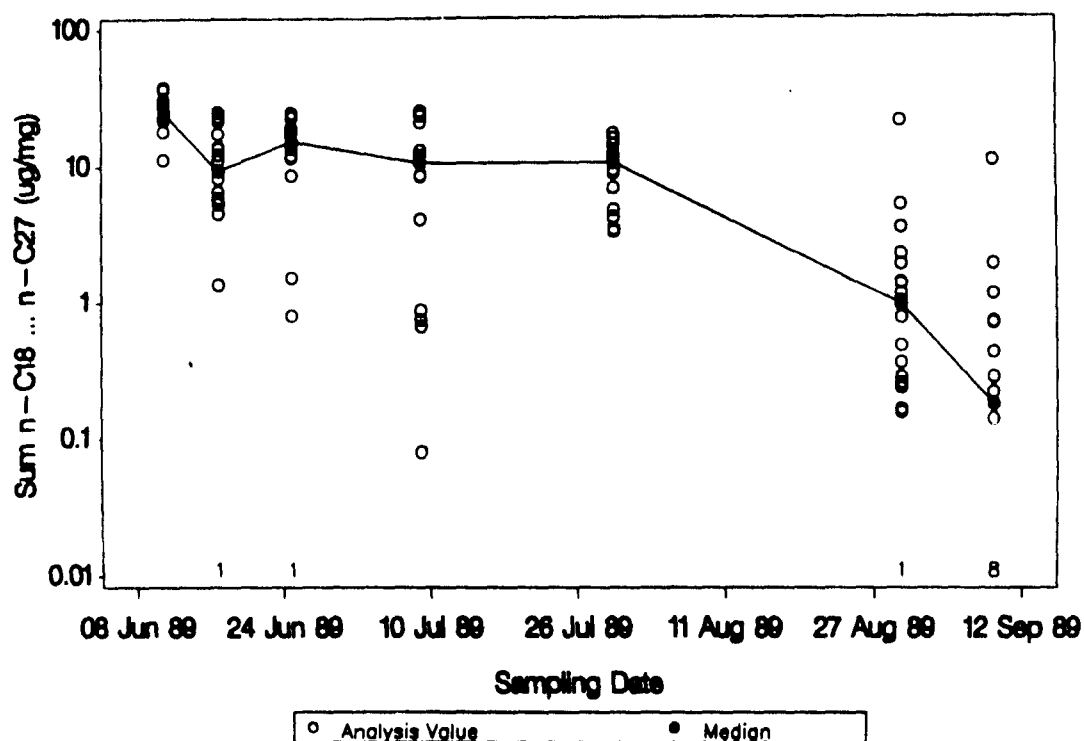
Figure 6.26. Change in Sum of Alkane Concentration nC18 to nC27 Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
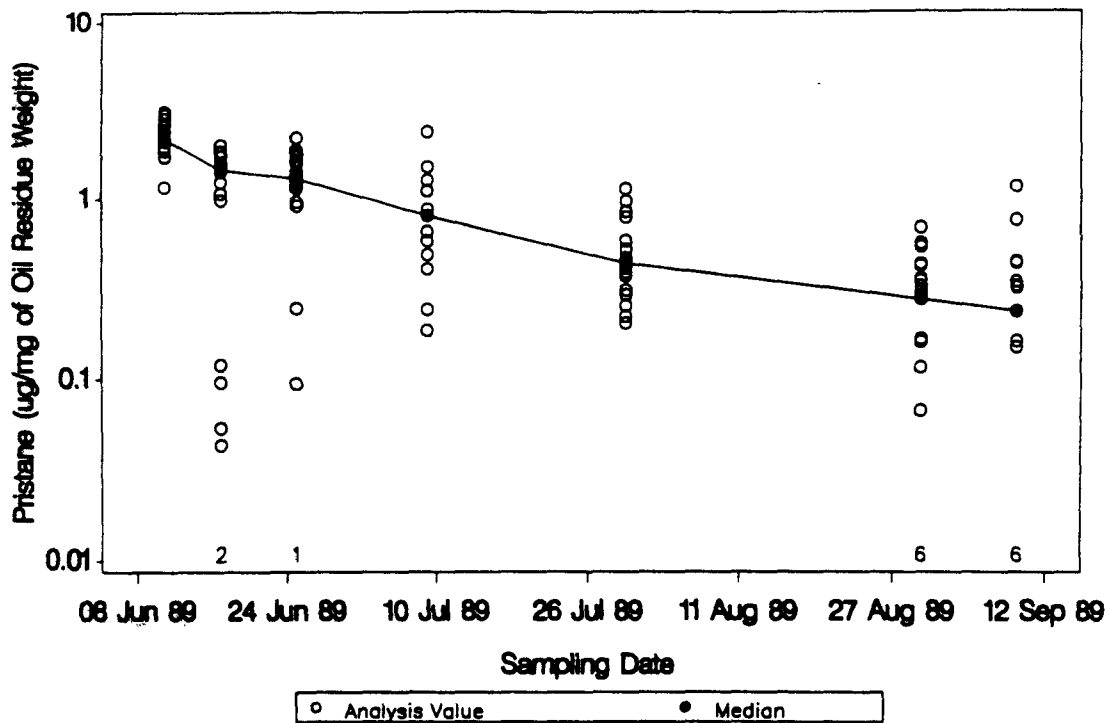
Figure 6.27. Change in Pristane Concentration Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
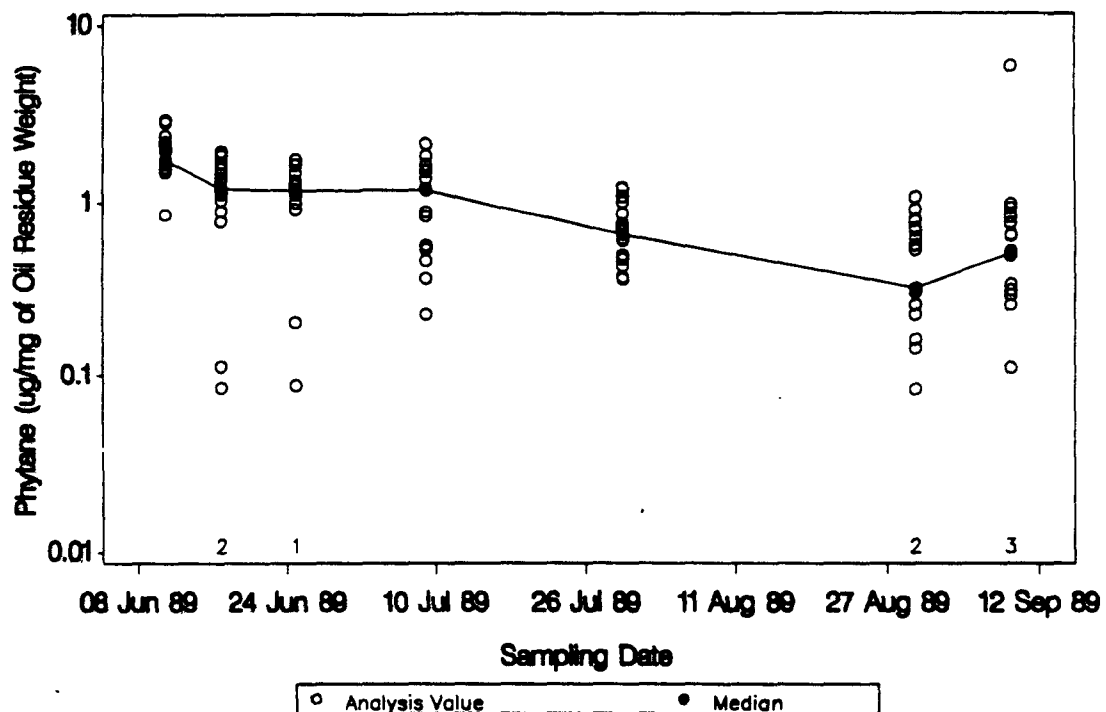
Figure 6.28. Change in Phytane Concentration Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

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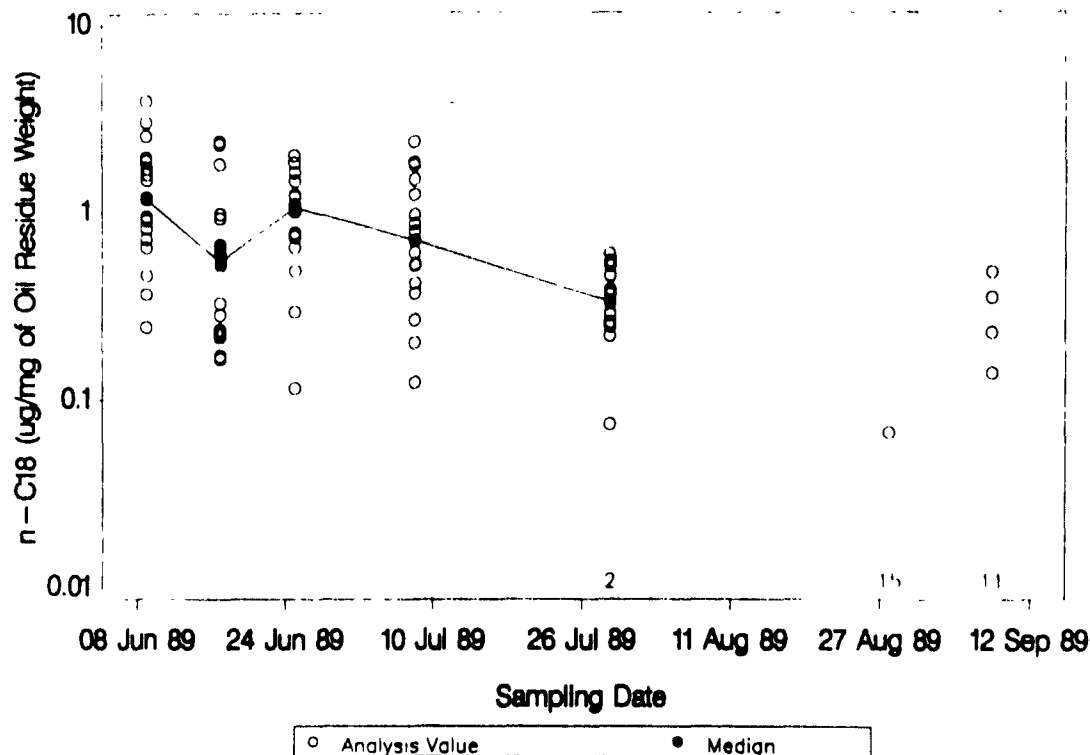
Figure 6.29. Change In nC18 Concentration Through Time for Seal Beach (INIPOL) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 6.30. Change In nC22 Concentration Through Time for Seal Beach (INIPOL) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 6.31. Change in nC27 Concentration Through Time for Seal Beach (INIPOL) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 6.32. Change in Sum of Alkane Concentration nC18 to nC27 Through Time for Seal Beach (INIPOL) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 6.33. Change in Pristane Concentration Through Time for Seal Beach (INIPOL) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 6.34. Change in Phytane Concentration Through Time for Seal Beach (INIPOL) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 6.35. Change In nC18/phytane Ratio Through Time for Seal Beach (Untreated Control) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 6.36. Change In nC18/phytane Ratio Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 6.37. Change in nC18/phytane Ratio Through Time for Seal Beach (INIPOL) at Snug Harbor (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
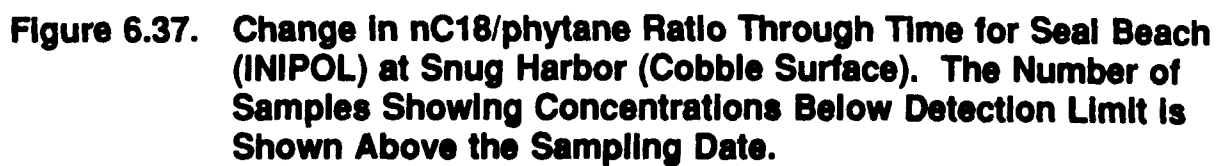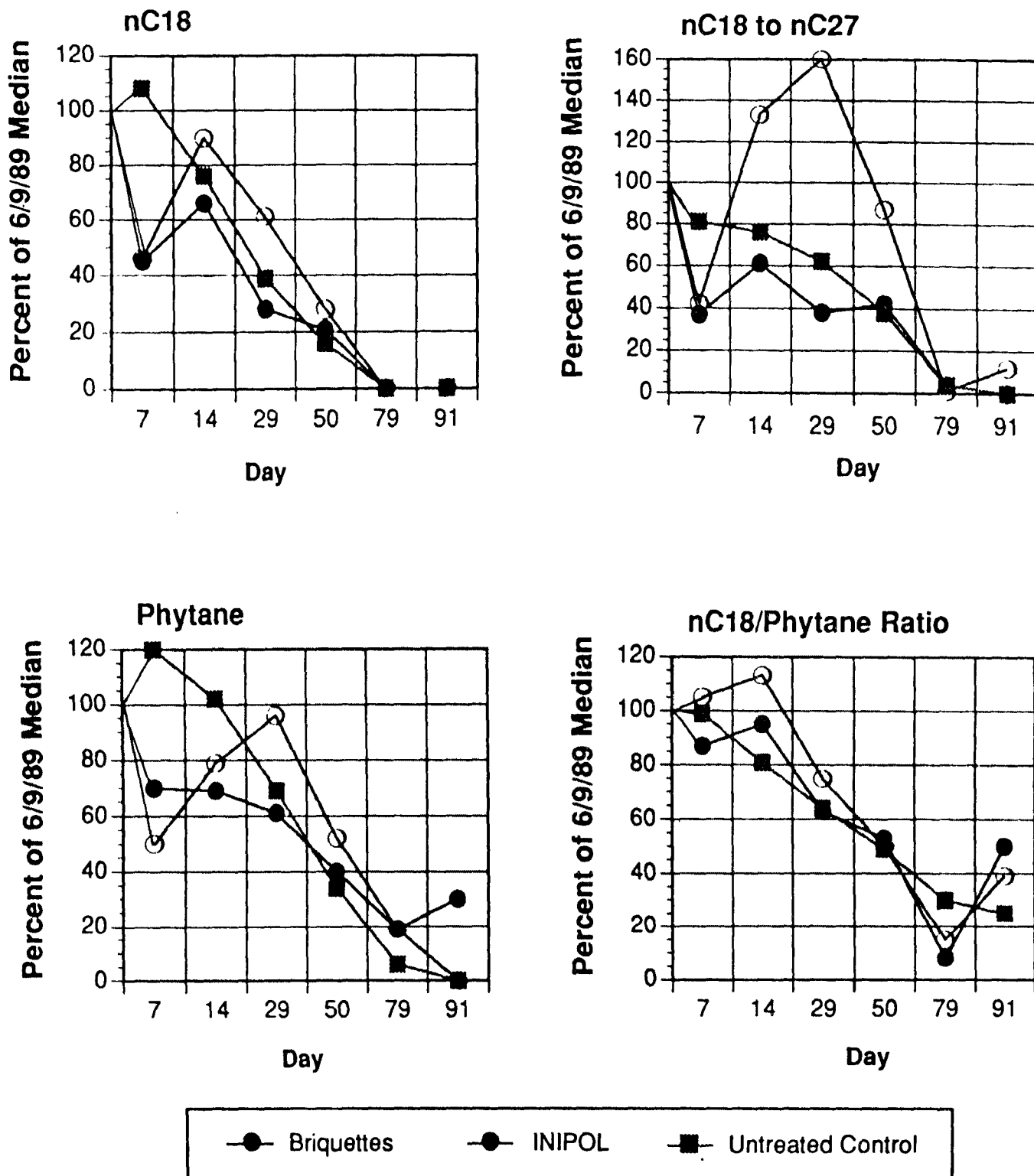
Figure 6.38. Change In the Median Residue Weight for Several Hydrocarbons Expressed as Percent of the 6/9/89 Median Over Time for the Briquette, INIPOL, and Untreated Control Beaches at Snug Harbor (Cobble Surface). All Variability Is not Shown Because the Actual Data Points are not Presented.

TABLE 6.7. CHANGE IN HYDROCARBON COMPOSITION THROUGH TIME AT SNUG HARBOR, EXPRESSED IN PERCENT OF THE MEDIAN CONCENTRATION OF INDIVIDUAL HYDROCARBONS ON THE 6/9 SAMPLING[a] (COBBLE SURFACE)

Alkane	Beach Code	6/17	6/25	7/8	7/29	8/26	9/9
nC18	B	45	66	28	21	0	0
	I	46	90	61	28	0	0
	C	108	76	39	16	0	0
nC19	B	40	65	36	31	4	4
	I	50	111	105	55	0	0
	C	92	72	53	25	0	0
nC20	B	32	63	42	40	2	0
	I	35	99	136	55	0	10
	C	92	65	64	33	0	0
nC21	B	37	64	40	43	1	0
	I	49	121	141	83	0	0
	C	83	76	71	36	0	0
nC22	B	42	61	42	46	5	0
	I	46	117	166	111	0	0
	C	75	73	69	40	0	0
nC23	B	46	56	38	39	4	0
	I	42	143	169	103	0	13
	C	90	89	79	42	7	0
nC24	B	43	58	40	47	0	0
	I	35	134	166	102	0	0
	C	80	90	72	47	0	0
nC25	B	40	57	41	54	0	0
	I	30	153	207	116	0	0
	C	74	84	70	50	0	0
nC26	B	43	55	39	49	0	0
	I	31	169	226	135	0	0
	C	68	74	67	44	2	0
nC27	B	42	55	43	49	2	0
	I	28	173	240	150	0	0
	C	72	102	98	61	4	0
nC18 to nC27	B	37	61	38	42	4	0
	I	42	133	160	87	1	12
	C	81	76	62	38	4	0
Phytane	B	70	69	61	40	19	30
	I	50	79	96	52	19	0
	C	120	102	69	34	6	0

[a] B = Briquette fertilizer-treated plot; I = INIPOL fertilizer-treated plot; C = Untreated Control

TABLE 6.8. NUMBER OF SAMPLES, OUT OF APPROXIMATELY 21 SAMPLES, TAKEN AT EACH SAMPLING TIME AT SNUG HARBOR, WITH ALKANE CONCENTRATION BELOW DETECTION LIMIT[a] (COBBLE SURFACE)

Alkane	Beach Code	6/9	6/17	6/25	7/8	7/29	8/26	9/9
nC18	B	0	1	2	3	0	11	16
	I	0	0	0	0	2	15	11
	C	0	0	1	0	3	18	18
nC19	B	0	1	1	0	0	5	8
	I	0	0	0	0	1	11	10
	C	0	0	0	0	1	12	16
nC20	B	0	1	1	3	0	8	15
	I	0	0	0	0	0	13	5
	C	0	0	0	0	0	15	18
nC21	B	0	1	1	1	0	10	13
	I	0	0	0	0	0	14	11
	C	0	0	0	0	0	13	18
nC22	B	0	1	1	1	0	4	13
	I	0	0	0	0	0	11	8
	C	0	0	0	0	0	12	15
nC23	B	0	1	1	2	0	7	10
	I	0	0	0	0	0	10	5
	C	0	0	0	0	0	8	16
nC24	B	0	1	1	3	0	12	16
	I	0	0	0	0	0	13	11
	C	0	1	0	0	0	12	15
nC25	B	0	1	1	2	0	12	16
	I	0	1	0	0	0	12	9
	C	0	1	0	0	0	11	16
nC26	B	0	1	1	2	0	13	17
	I	0	1	0	0	0	10	9
	C	0	1	0	0	0	10	15
nC27	B	0	1	2	3	0	10	17
	I	0	2	0	0	0	13	10
	C	0	3	0	0	0	10	14
nC18 to nC27	B	0	1	1	0	0	1	8
	I	0	0	0	0	0	8	4
	C	0	0	0	0	0	6	13
Phytane	B	0	2	1	0	0	2	3
	I	0	0	0	0	2	5	8
	C	0	0	0	1	2	9	11

[a] B = Briquette fertilizer-treated plot; I = INIPOL fertilizer-treated plot; C = Untreated Control

TABLE 6.9. MEDIAN VALUES AND STATISTICAL COMPARISONS OF OIL RESIDUE WEIGHTS FOR SUMMED ALKANES IN COBBLE SURFACE SAMPLES FROM THE DIFFERENT BEACH TREATMENTS AT SNUG HARBOR

Median Values (% of 6/9/89 Median)

Sampling Date	Day	Untreated Control	Briquettes	INIPOL
June 9	0	17.0	25.1	9.9
June 17	8	13.7 (81)	9.4 (37)	4.2 (42)
June 25	16	12.9 (76)	15.3 (61)	13.2 (133)
July 8	29	10.6 (62)	9.6 (38)	15.9 (160)
July 29	50	6.4 (38)	10.6 (42)	8.6 (87)
August 26	78	0.71 (4)	0.95 (4)	0.05 (1)
September 9	92	0 (0)	0.18 (0)	1.2 (12)

Mann-Whitney Test Results[a]

Sampling Date	Briquettes vs. INIPOL	Briquettes vs. Untreated Control	INIPOL vs. Untreated Control
June 9	B > I	B > C	Same
June 17	B > I	Same	Same
June 25	B > I	Same	Same
July 8	Same	Same	Same
July 29	Same	B > C	I > C
August 26	B > I	B > C	Same
September 9	B < I	Same	I > C

[a] 95 Percent Confidence Level

TABLE 6.10. RATE ANALYSIS OF NATURAL LOG-TRANSFORMED OIL RESIDUE
WEIGHTS FOR SUMMED ALKANES IN COBBLE SURFACE SAMPLES VERSUS TIME
FOR TEST BEACHES AT SNUG HARBOR

| Beach | Slope (Std. Dev.) | Significance of Slope Greater than Zero | | |
		N	T-value	p
Briquettes	-0.017 (0.006)	89	-2.9	0.005
INIPOL	-0.001 (0.005)	100	0.26	0.80
Untreated Control	-0.011 (0.005)	84	-2.4	0.02

It is difficult to explain the results for the plots treated with INIPOL (Figures 6.29 to 6.34 and 6.38). Following a rather dramatic decrease in alkane concentration (especially for the higher molecular weight alkanes) during the 8 days after fertilizer application, it appears that the concentrations actually increased significantly. Although it is possible that biodegradation of the fertilizer components may have produced intermediates that confounded the GC analysis of the hydrocarbons, it seems very unlikely that these products would be extracted with the methylene chloride procedure used in the analyses. If the presence of INIPOL caused other less degraded oil to be mixed in with the original oil, similar increases in residue weight would be expected. This was not the case. Finally, it is possible that chemical analysis of the samples taken on June 25 was done improperly. Again, there was nothing to suggest that this occurred, and samples on subsequent dates also showed elevated concentrations of the normal alkanes. Thus, these results can not be explained and may have been obscuring any changes that corresponded with the decreases in oil residue weight. The dramatic increases in hydrocarbon decay rates following the July 29 sampling were also seen on the INIPOL-treated plot. The extent of alkane removal appeared to be greatest on the INIPOL-treated plot.

A further comparison of the decay rates between the normal alkanes and the branched alkanes (pristane and phytane) for all plots (Figures 6.21, 6.22, 6.27, 6.28, 6.33, 6.34 and 6.38) reveals similar

trends, but with several notable exceptions. First, significant decreases in the branched alkane concentrations occurred in all cobble surface samples. If these decreases were due to biodegradation, it suggests that the branched alkanes were not as conserved as originally expected, and any ratioing of the normal alkanes to the branched alkanes will give conservative estimates of biodegradation (see below).

Second, on the untreated control plot decreases in pristane and phytane concentrations (Figures 6.21 and 6.22) following the initial lag were surprisingly rapid. In fact, the decreases equaled those for the nC18 alkane and surpassed the decreases observed for the higher molecular weight alkanes. This represents possibly a unique biodegradation capability on the untreated control plot.

Finally, pristane and phytane concentrations in samples taken from the briquette fertilizer-treated plot (Figures 6.27 and 6.28) did not show the dramatic change in decay rate following the July 29 sampling observed with many of the other alkanes in the same samples. This implies that the events responsible for this accelerated decay may have been due to biodegradation.

Decreases in the nC18/phytane ratios for the two treated plots and the untreated control (Figures 6.35-6.37 and 6.38) strongly suggest that biodegradation was affecting the changes in hydrocarbon composition in all cases. No other physical or chemical process will differentially effect changes in the concentration of two alkanes that chemically behave very similarly (their boiling points are very close, causing them to chromatograph very close to one another in a GC column). Statistical analysis of the decay rates for the ratios is shown in Tables 6.11 and 6.12. All rates were significantly different from zero at the 95% confidence interval, and the decay rate of the ratios was slightly faster on the two fertilizer-treated beaches. Thus, changes in alkane composition suggest that the addition of the fertilizers caused a small but significant enhancement of biodegradation, and this enhanced biodegradation activity was probably largely responsible for the observed fertilizer-induced changes in the oil residue weights noted in the previous section.

b) Mixed Sand and Gravel Samples Under Cobble

Changes through time in the concentration of selected normal alkanes (nC18, nC22, and nC27), the sum of normal alkanes (nC18 to nC27), the branched alkanes pristane and phytane, and the nC18/phytane ratios in mixed sand and gravel samples under cobble from treated and untreated control plots, are shown in Figures 6.39 to 6.59. For comparative purposes, Tables 6.13 and 6.14

TABLE 6.11. MEDIAN VALUES AND STATISTICAL COMPARISONS OF THE nC18/PHYTANE RATIO IN COBBLE SURFACE SAMPLES FROM DIFFERENT BEACH TREATMENTS AT SNUG HARBOR

Median Values (Number of above detection limit values for nC18 and phytane)

Sampling Date	Day	Untreated Control (n)	Briquettes (n)	INIPOL (n)
June 9	0	1.06 (19)	1.46 (18)	1.24 (20)
June 17	8	1.05 (19)	1.27 (17)	1.30 (19)
June 25	16	0.86 (16)	1.38 (18)	1.40 (18)
July 8	29	0.68 (9)	0.92 (11)	0.93 (19)
July 29	50	0.52 (15)	0.77 (19)	0.62 (16)
August 26	78	0.32 (3)	0.11 (9)	0.18 (1)
September 9	92	0.27 (1)	0.73 (2)	0.48 (4)

Mann-Whitney Test Results[a]

Sampling Date	Briquettes vs. INIPOL	Briquettes vs. Untreated Control	INIPOL vs. Untreated Control
June 9	B > I	B > C	I > C
June 17	Same	Same	Same
June 25	Same	B > C	I > C
July 8	Same	Same	Same
July 29	B > I	B > C	I > C
August 26	NA	B < C	NA
September 9	NA	NA	NA

[a] 95 Percent Confidence Level
NA = Insufficient data to calculate a statistic valid at the 95% confidence level

TABLE 6.12. RATE ANALYSIS OF nC18/PHYTANE RATIOS IN COBBLE SURFACES
VERSUS TIME FOR TEST BEACHES AT SNUG HARBOR

| Beach | Slope (Std. Dev.) | Significance of Slope Greater than Zero | | |
		N	T-value	p[a]
Briquettes	-0.012 (0.0015)	94	-7.9	0.0001
INIPOL	-0.011 (0.0016)	101	-7.2	0.0001
Untreated Control	-0.009 (0.0013)	82	-6.9	0.0001

[a] All slopes are statistically significant at the 95 percent confidence level

TABLE 6.13. CHANGE IN HYDROCARBON COMPOSITION THROUGH TIME AT SNUG HARBOR, EXPRESSED IN PERCENT OF THE MEDIAN CONCENTRATION OF INDIVIDUAL HYDROCARBONS ON THE 6/9 SAMPLING[a] (MIXED SAND AND GRAVEL UNDER COBBLE)

Alkane	Beach Code	6/17	6/25	7/8	7/29	8/26	9/9
nC18	B	58	31	13	14	5	14
	I	97	56	45	31	20	39
	C	106	0	44	38	24	55
nC19	B	54	31	14	15	2	13
	I	80	48	37	28	17	30
	C	107	0	43	40	30	54
nC20	B	50	46	13	19	0	0
	I	74	40	42	30	11	22
	C	152	0	72	71	30	44
nC21	B	56	36	18	15	5	12
	I	88	57	35	33	17	39
	C	123	0	58	52	39	56
nC22	B	62	39	19	25	8	13
	I	89	57	41	42	17	29
	C	171	0	111	85	59	62
nC23	B	68	48	19	22	15	19
	I	76	46	42	35	26	19
	C	92	0	64	56	46	66
nC24	B	69	40	18	27	0	17
	I	100	55	41	43	17	1
	C	79	0	65	52	0	69
nC25	B	87	50	20	29	13	25
	I	95	61	38	38	13	0
	C	90	0	45	47	25	74
nC26	B	80	57	21	26	0	10
	I	112	66	23	40	17	0
	C	125	212	60	63	0	0
nC27	B	89	50	24	31	0	2
	I	172	67	40	37	16	0
	C	181	0	71	68	35	36
nC18 to nC27	B	67	39	16	23	7	13
	I	97	55	38	39	18	23
	C	112	59	65	61	35	59
Phytane	B	86	58	26	27	12	21
	I	81	60	49	31	21	22
	C	93	56	55	34	19	42

[a] B = Briquette fertilizer-treated plot; I = INIPOL fertilizer-treated plot; C = Untreated Control

TABLE 6.14. NUMBER OF SAMPLES, OUT OF APPROXIMATELY 21 SAMPLES, TAKEN AT EACH SAMPLING TIME AT SNUG HARBOR, WITH ALKANE CONCENTRATION BELOW DETECTION LIMIT[a] (MIXED SAND AND GRAVEL UNDER COBBLE)

Alkane	Beach Code	6/9	6/17	6/25	7/8	7/29	8/26	9/9
nC18	B	0	2	3	0	1	9	5
	I	2	2	4	0	0	0	5
	C	1	1	12	4	4	8	6
nC19	B	0	2	3	0	1	10	5
	I	2	2	2	1	0	0	5
	C	1	1	12	3	3	8	5
nC20	B	0	2	4	0	1	12	8
	I	2	2	3	0	0	4	6
	C	1	2	15	3	3	8	6
nC21	B	0	2	3	0	1	8	5
	I	2	3	2	0	0	1	5
	C	1	3	15	1	3	8	6
nC22	B	0	2	3	0	0	8	6
	I	2	3	2	0	0	1	5
	C	1	2	15	0	2	8	8
nC23	B	0	2	2	0	0	5	3
	I	2	3	2	1	0	0	6
	C	1	4	14	1	1	5	4
nC24	B	0	2	4	0	0	11	6
	I	2	3	3	3	0	2	9
	C	1	4	17	2	3	13	7
nC25	B	0	1	4	0	0	8	4
	I	2	3	3	0	0	4	10
	C	1	4	15	1	2	9	5
nC26	B	0	2	4	2	2	12	6
	I	2	4	5	3	0	6	10
	C	1	4	6	3	4	13	9
nC27	B	0	2	4	1	1	13	7
	I	3	3	6	3	0	6	10
	C	2	4	16	3	3	8	8
nC18 to nC27	B	0	1	2	0	0	5	1
	I	1	2	1	0	0	0	5
	C	1	0	2	0	0	3	2
Phytane	B	0	1	3	0	0	8	2
	I	1	2	1	0	0	0	5
	C	0	1	6	1	3	9	5

[a] B = Briquette fertilizer-treated plot; I = INIPOL fertilizer-treated plot; C = Untreated Control
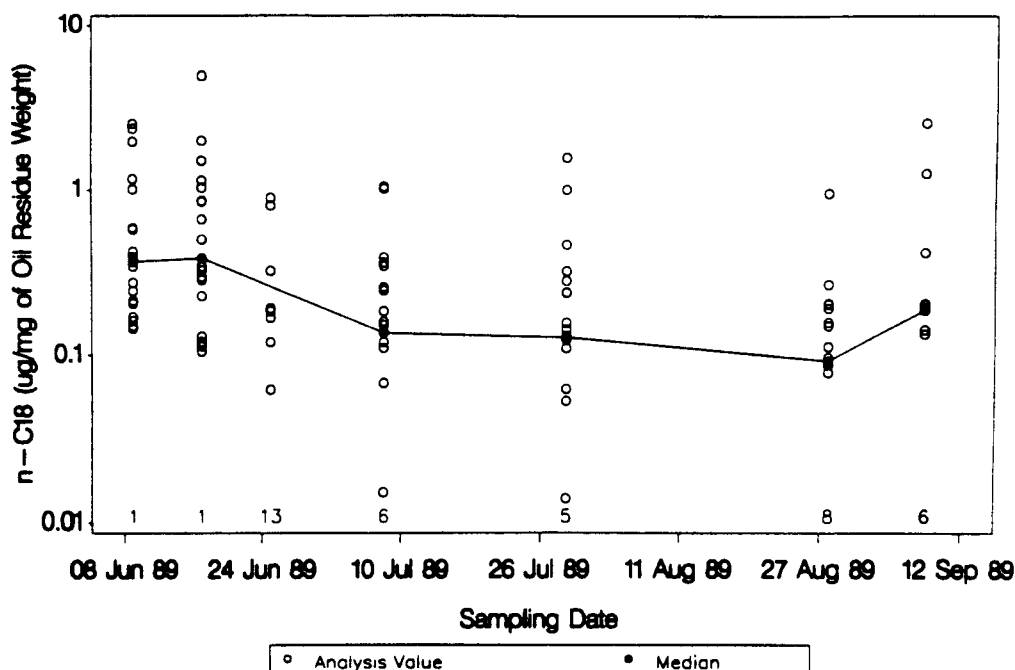
Figure 6.39. Change in nC18 Concentration Through Time for Seal Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
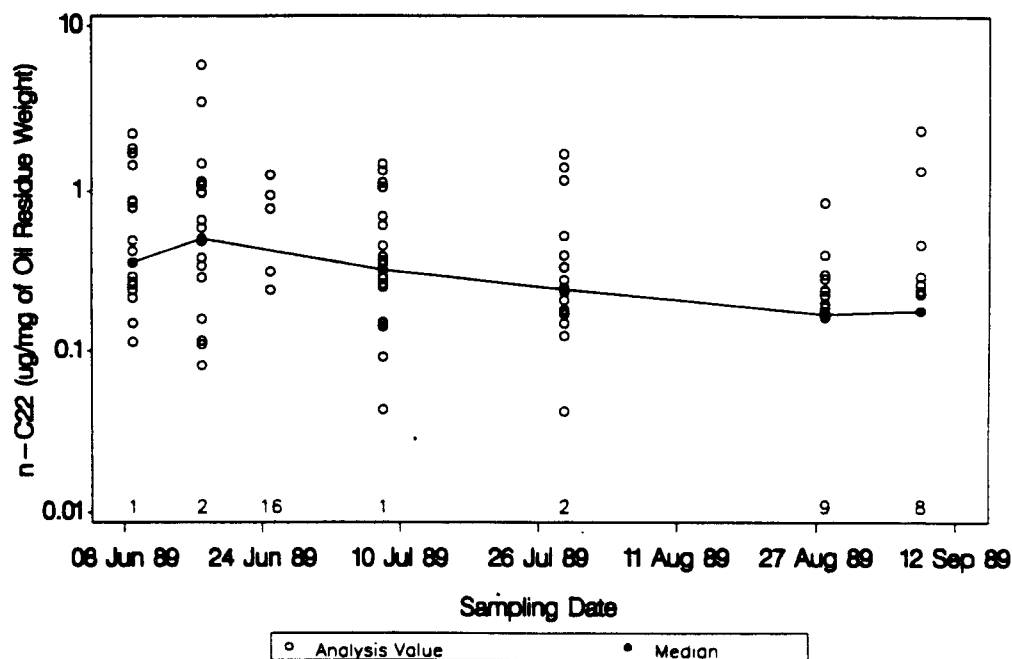
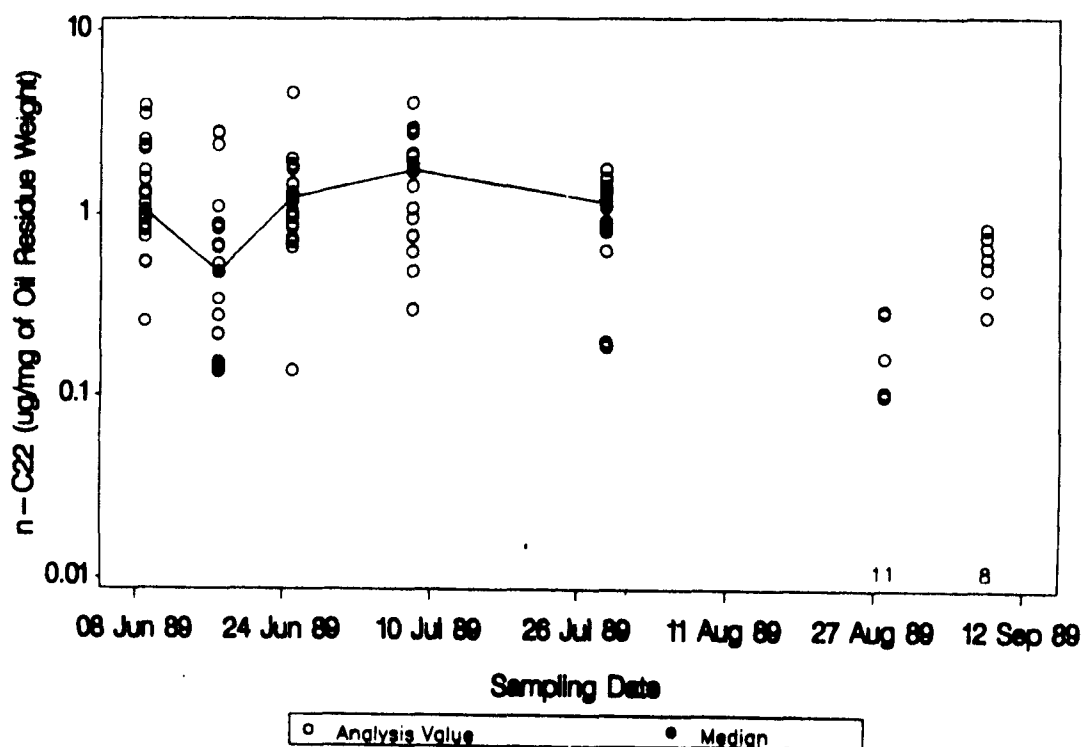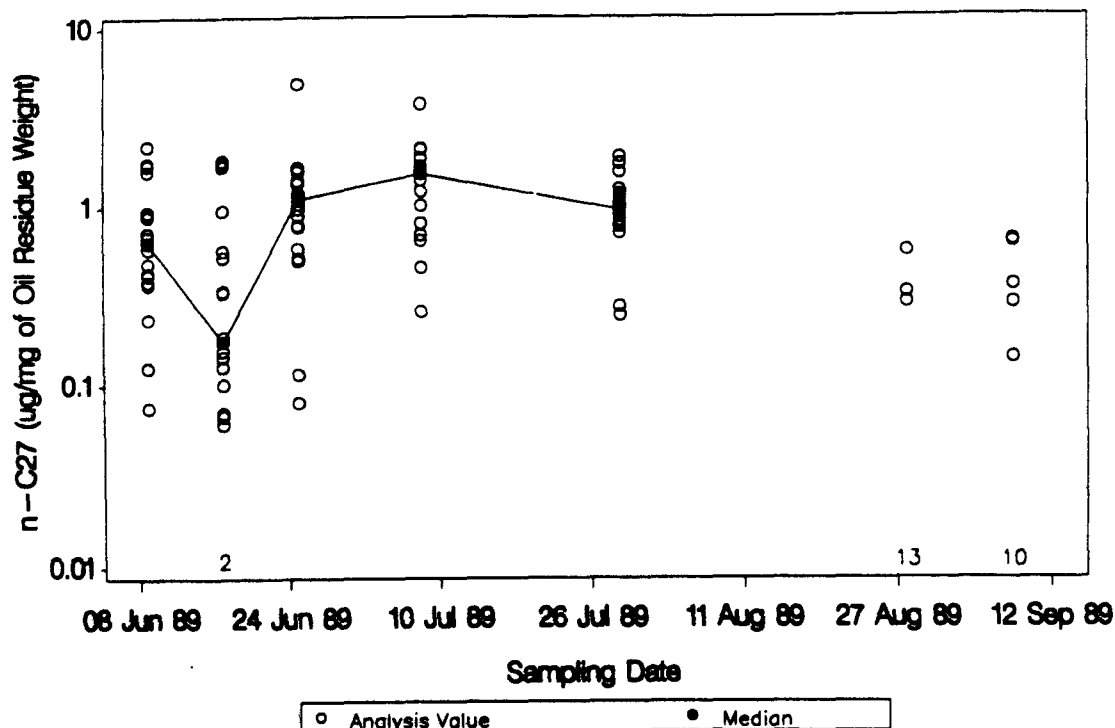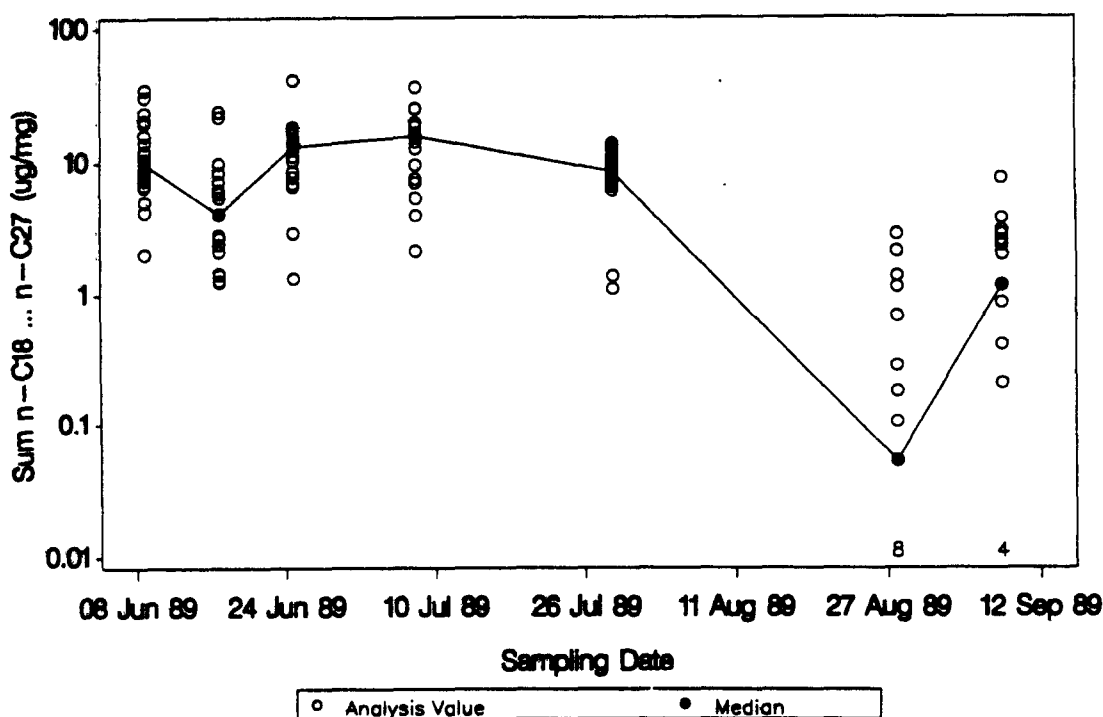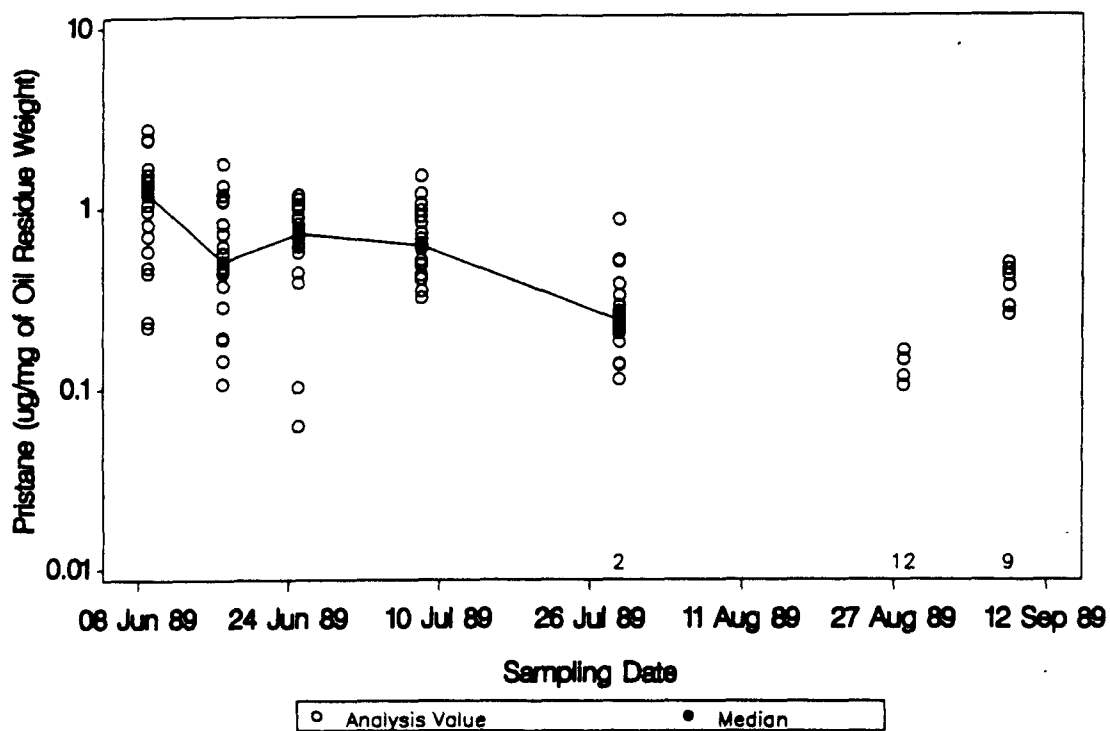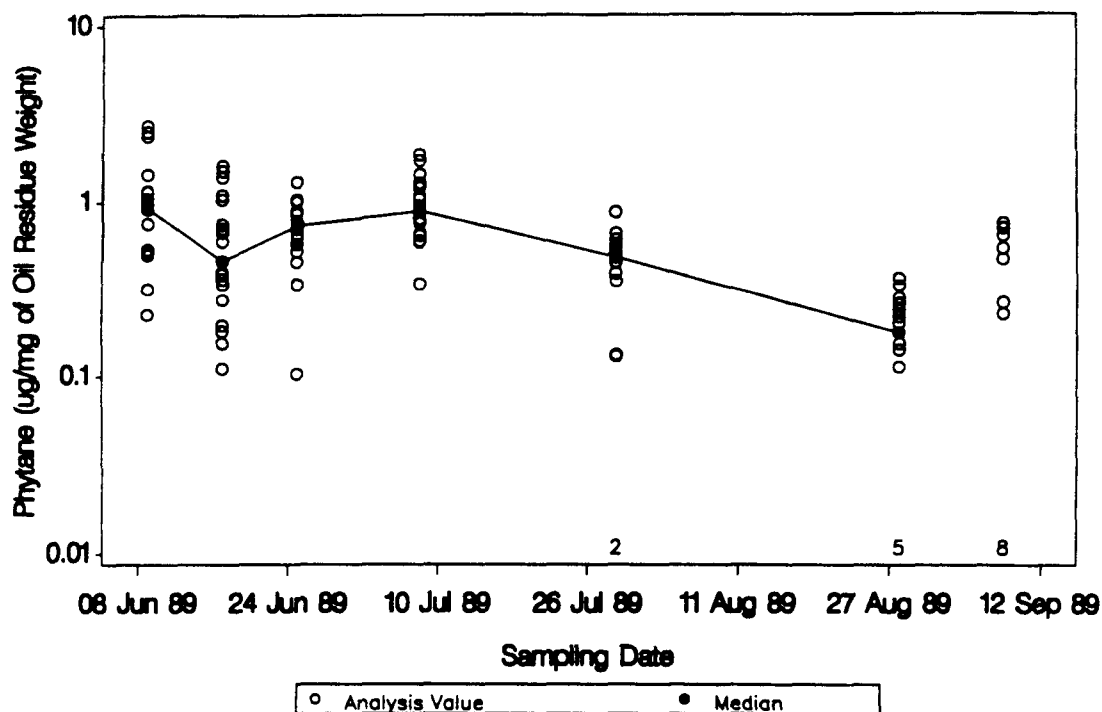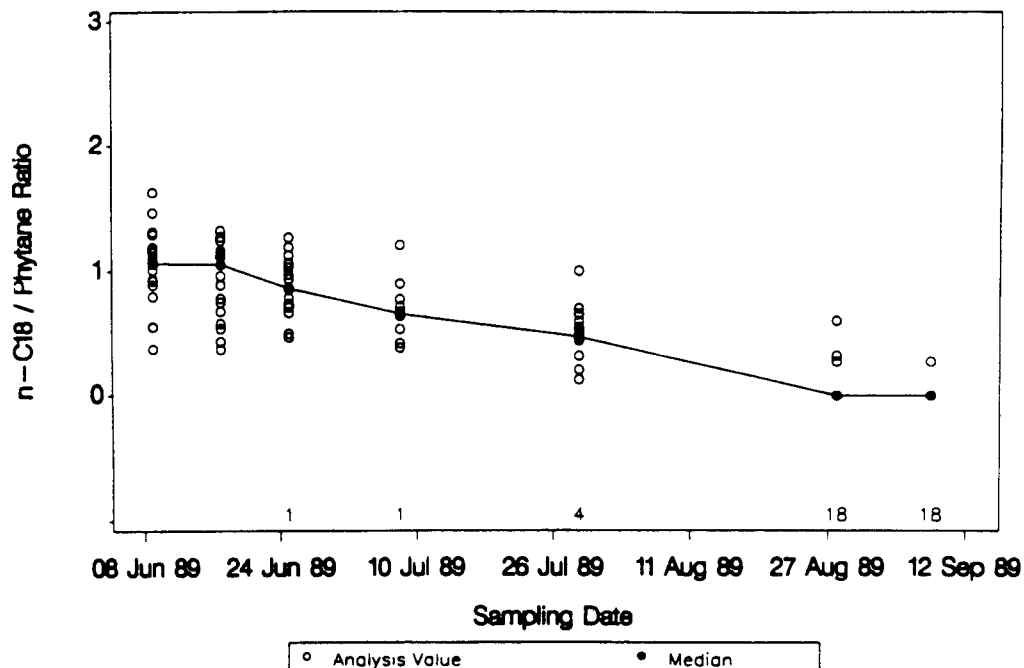**Figure 6.40.** Change in nC22 Concentration Through Time for Seal Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
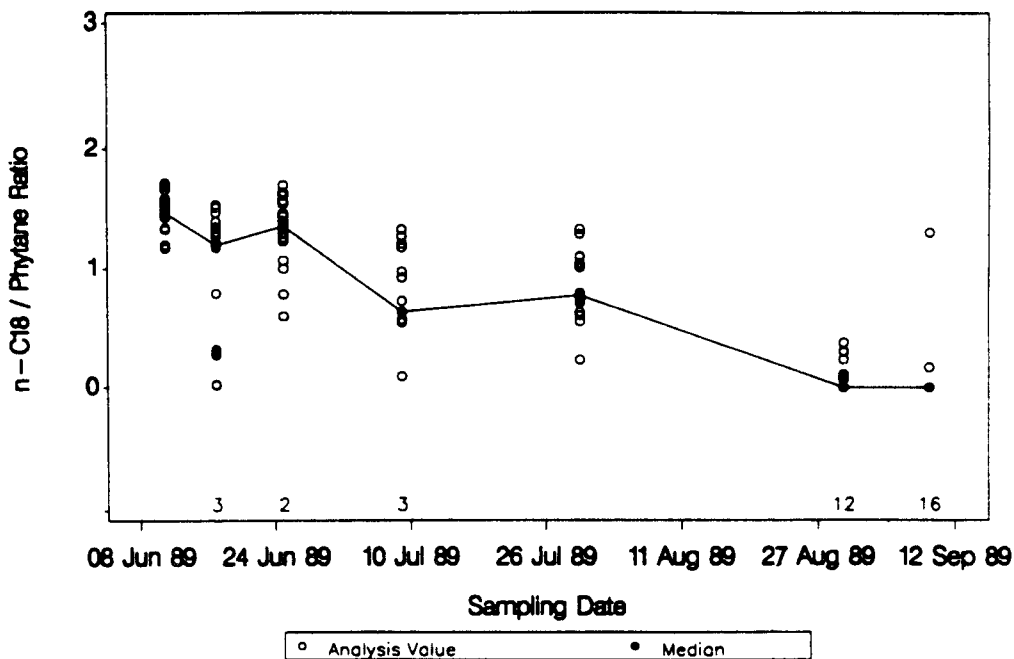
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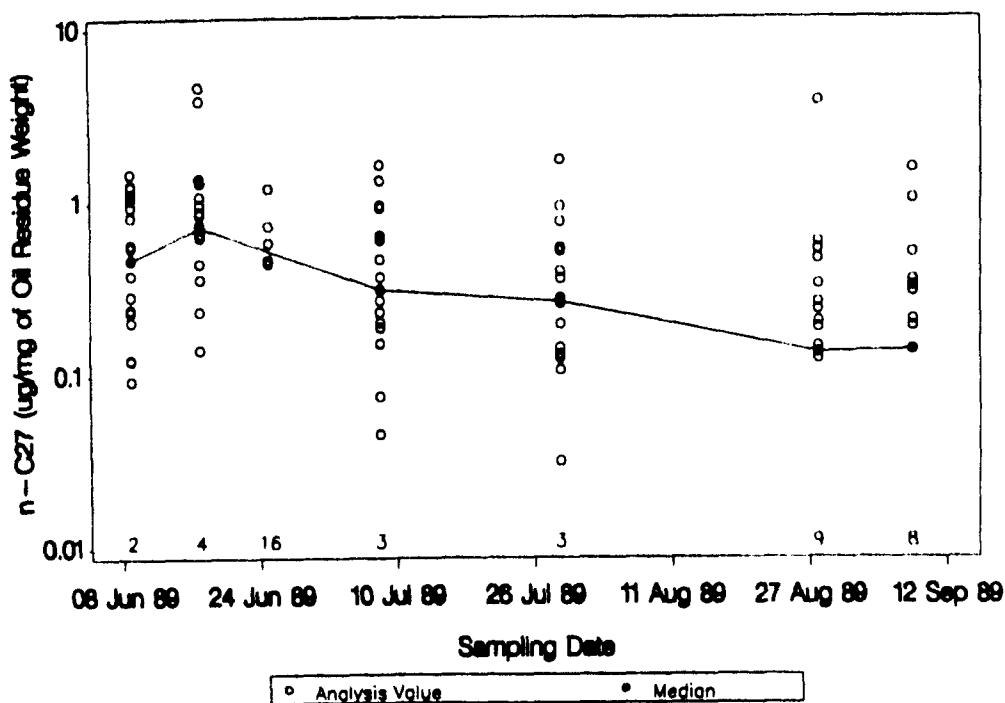
Figure 6.41. Change In nC27 Concentration Through Time for Seal Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

Figure 6.42. Change In the Sum of Alkane Concentration nC18 to nC27 Through Time for Seal Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

**Figure 6.43.** Change In Pristane Concentration Through Time for Seal Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

Figure 6.44. Change In Phytane Concentration Through Time for Seal Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

Figure 6.45. Change in nC18 Concentration Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 6.46. Change in nC22 Concentration Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

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Figure 6.47. Change in nC27 Concentration Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 6.48. Change in the Sum of Alkane Concentration nC18 to nC27 Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
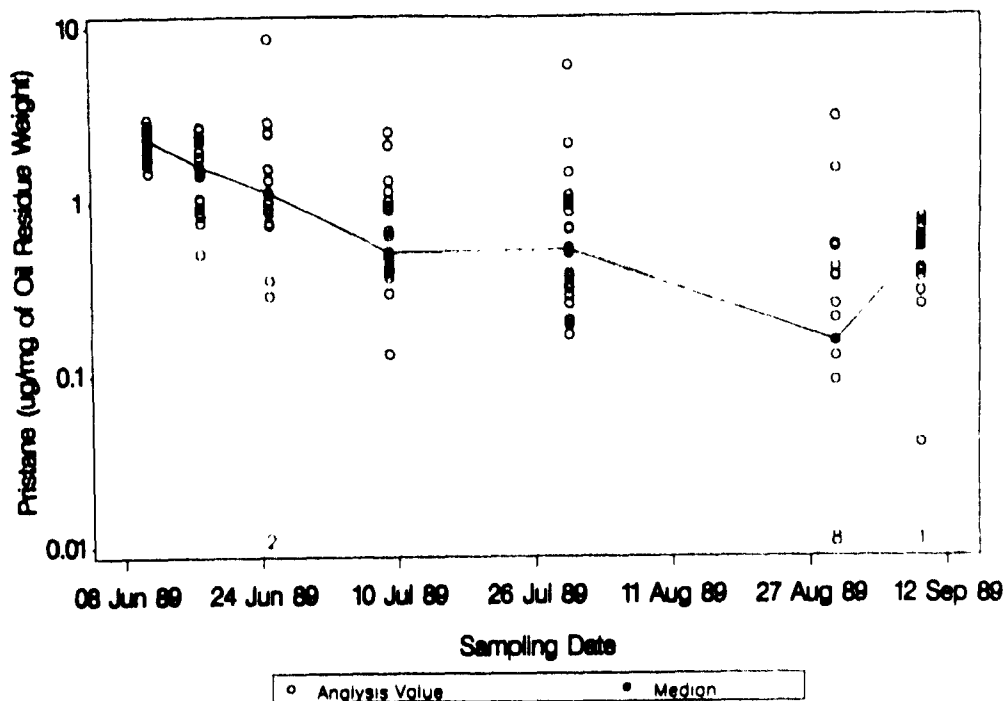
Figure 6.49. Change in Pristane Concentration Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

Figure 6.50. Change in Phytane Concentration Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

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Figure 6.51. Change In nC18 Concentration Through Time for Seal Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

Figure 6.52. Change In nC22 Concentration Through Time for Seal Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

Figure 6.53. Change In nC27 Concentration Through Time for Seal Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

Figure 6.54. Change In the Sum of Alkane Concentration nC18 to nC27 Through Time for Seal Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

Figure 6.55. **Change in Pristane Concentration Through Time for Seal Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.**

Figure 6.56. **Change in Phytane Concentration Through Time for Seal Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.**

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Figure 6.57. Change in nC18/Phytane Ratio Through Time for Seal Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 6.58. Change in nC18/Phytane Ratio Through Time for Seal Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

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Figure 6.59. Change in nC18/Phytane Ratio Through Time for Seal Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel Under Cobble). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

summarize the percent change in the medians of individual hydrocarbons and the number of samples with a hydrocarbon concentration of zero (below detection limits) with each sampling period, respectively. Figure 6.60 provides a graphical representation of the percent change in the medians for several hydrocarbons.

The results for the mixed sand and gravel under cobble represent the second dramatic effect of the fertilizer application. Changes in concentrations of all alkanes seemed to follow the same general trend; decay with time was relatively first-order, with the fastest rates seen on the fertilizer briquette-treated plot and the slowest rates on the untreated control. Rates on the INIPOL fertilizer-treated plot were generally in-between. The effect of the fertilizers can be shown statistically if the summed alkanes are used as an exemplary indicator of the trends. As shown in Tables 6.15 and 6.16, all decay rates were significantly different from zero at the 95% confidence level and the INIPOL and briquette fertilizers enhanced the rates two- and three-fold, respectively. These rates were also significantly different from each other. Thus, despite high variability in the samples, differences in decay rates were large enough to see a fertilizer effect.

Overall, decay in alkane concentrations in mixed sand and gravel samples taken from under cobble was much less complex than that seen with the cobble surface samples. There were neither large decreases in concentration during the latter part of the sampling period, nor apparent increases in concentrations observed in samples from the INIPOL fertilizer-treated plots. Decays for most alkanes commenced immediately after fertilizer application, but in contrast to the cobble surface samples, the decay continued and did not appear to level off. A much shorter lag in hydrocarbon concentration change occurred in the mixed sand and gravel samples compared to the untreated control plot cobble surface samples. The only peculiar result was a fairly consistent increase in alkane concentrations the last sampling. This may have been related to other clean-up operations occurring in Snug Harbor at that time.

An examination of the changes in the nC18/phytane ratios (Figures 6.57, 6.58, and 6.59) showed similar trends, with fastest change on the briquette fertilizer-treated plot, slowest change on the untreated control, and intermediate change on the INIPOL fertilizer-treated plot. However, the curves are complicated because of a lack of any significant change in the ratios during the first 2 to 4 samplings. This is reflected in the statistical analysis of the decay curves shown in Tables 6.17 and 6.18, and is due to the same disappearance rate for both the normal alkane and the branched alkane, a process that could possibly be attributed to fate processes other than biodegradation. However,

Figure 6.60. Change in the Median Residue Weight for Several Hydrocarbons Expressed as Percent of the 6/9/89 Median Over Time for the Briquette, INIPOL, and Untreated Control Beaches at Snug Harbor (Mixed Sand and Gravel Under Cobble). All Variability is not Shown Because the Actual Data Points are not Presented.

TABLE 6.15. MEDIAN VALUES AND STATISTICAL COMPARISONS OF OIL RESIDUE WEIGHTS FOR SUMMED ALKANES IN MIXED SAND AND GRAVEL FROM DIFFERENT BEACH TREATMENTS AT SNUG HARBOR

Median Values (% of 6/9/89 Median)

Sampling Date	Day	Untreated Control	Briquettes	INIPOL
June 9	0	3.5	15.9	7.5
June 17	8	3.9 (112)	10.7 (67)	7.3 (97)
June 25	16	2.1 (60)	6.2 (39)	4.1 (55)
July 8	29	2.3 (65)	2.6 (16)	2.8 (38)
July 29	50	2.1 (60)	3.7 (23)	2.9 (39)
August 26	78	1.2 (35)	1.0 (7)	1.4 (18)
September 9	92	2.1 (60)	2.0 (13)	1.7 (23)

Mann-Whitney Test Results[a]

Sampling Date	Briquettes vs. INIPOL	Briquettes vs. Untreated Control	INIPOL vs. Untreated Control
June 9	B > I	B > C	I > C
June 17	Same	B > C	I > C
June 25	B > I	B > C	I > C
July 8	Same	Same	Same
July 29	Same	Same	Same
August 26	Same	Same	Same
September 9	Same	Same	Same

[a] 95 Percent Confidence Level

TABLE 6.16. RATE ANALYSIS OF NATURAL LOG-TRANSFORMED OIL RESIDUE
WEIGHTS FOR SUMMED ALKANES IN MIXED SAND AND GRAVEL
UNDER COBBLE VERSUS TIME FOR TEST BEACHES AT SNUG HARBOR

| Beach | Slope (Std. Dev.) | Significance of Slope Greater than Zero | | |
		N	T-value	p
Briquettes	-0.032 (0.005)	99	-6.8	0.0001
INIPOL	-0.023 (0.005)	96	-4.3	0.0001
Untreated Control	-0.013 (0.006)	94	-2.2	0.03

TABLE 6.17. MEDIAN VALUES AND STATISTICAL COMPARISONS OF
THE nC18/PHYTANE RATIO IN MIXED SAND AND GRAVEL FROM DIFFERENT
BEACH TREATMENTS[a] AT SNUG HARBOR

Median Values (Number of above detection limit values for nC18 and phytane)

Sampling Date	Day	Untreated Control (n)	Briquettes (n)	INIPOL (n)
June 9	0	0.53 (18)	1.00 (19)	0.69 (19)
June 17	8	0.47 (18)	0.73 (19)	0.76 (18)
June 25	16	0.34 (8)	0.70 (17)	0.58 (14)
July 8	29	0.49 (15)	0.56 (21)	0.52 (20)
July 29	50	0.63 (15)	0.48 (20)	0.57 (21)
August 26	78	0.53 (11)	0.79 (11)	0.64 (20)
September 9	92	0.58 (11)	0.52 (10)	0.74 (13)

Mann-Whitney Test Results[a]

Sampling Date	Briquettes vs. INIPOL	Briquettes vs. Untreated Control	INIPOL vs. Untreated Control
June 9	B > I	B > C	I > C
June 17	Same	Same	I > C
June 25	Same	B > C	I > C
July 8	Same	Same	Same
July 29	B < I	Same	Same
August 26	B > I	Same	Same
September 9	B < I	Same	Same

[a] 95 Percent Confidence Level

TABLE 6.18. RATE ANALYSIS OF THE nC18/PHYTANE RATIO IN MIXED SAND AND
GRAVEL SAMPLES VERSUS TIME FOR TEST BEACHES AT SNUG HARBOR

| Beach | Slope (Std. Dev.) | Significance of Slope Greater than Zero | | |
		N	T-value	p[a]
Briquettes	-0.0014 (0.0012)	117	-1.23	0.22
INIPOL	-0.0003 (0.0009)	125	-0.37	0.71
Untreated Control	-0.005 (0.006)	96	-0.82	0.42

[a] None of these slopes are significantly different from zero at the 95 percent confidence level

since the application of nutrients from the fertilizer briquettes did not have a chemical or physical effect on the oil, it is highly likely that all of the observed changes in oil composition were due to biodegradation. It is interesting that the large changes in composition were not accompanied by changes in oil residue weight, as described in the last section. It is possible that the degraded oil is much more easily removed from the cobble surfaces than the mixed sand and gravel underneath.

Mixed Sand and Gravel Plots (Otter and Eagle Beaches)

Changes through time in the concentration of selected normal alkanes (nC18, nC22, and nC27), the sum of normal alkanes (nC18 to nC27), the branched alkanes pristane and phytane, and the nC18/phytane ratios in samples from the mixed sand and gravel plots (no cobble on the surface) are shown in Figures 6.61 to 6.81. As indicated above, all values of hydrocarbon concentration were normalized to the weight of oil in the extracted sample. In all cases, values below detection limits were treated as zero.

The effect of fertilizer application was most apparent on the plot treated with the fertilizer briquettes. Over the first 29 days there was a greater decrease in the individual hydrocarbon concentrations on the briquette fertilizer-treated plot relative to the untreated control. Using the
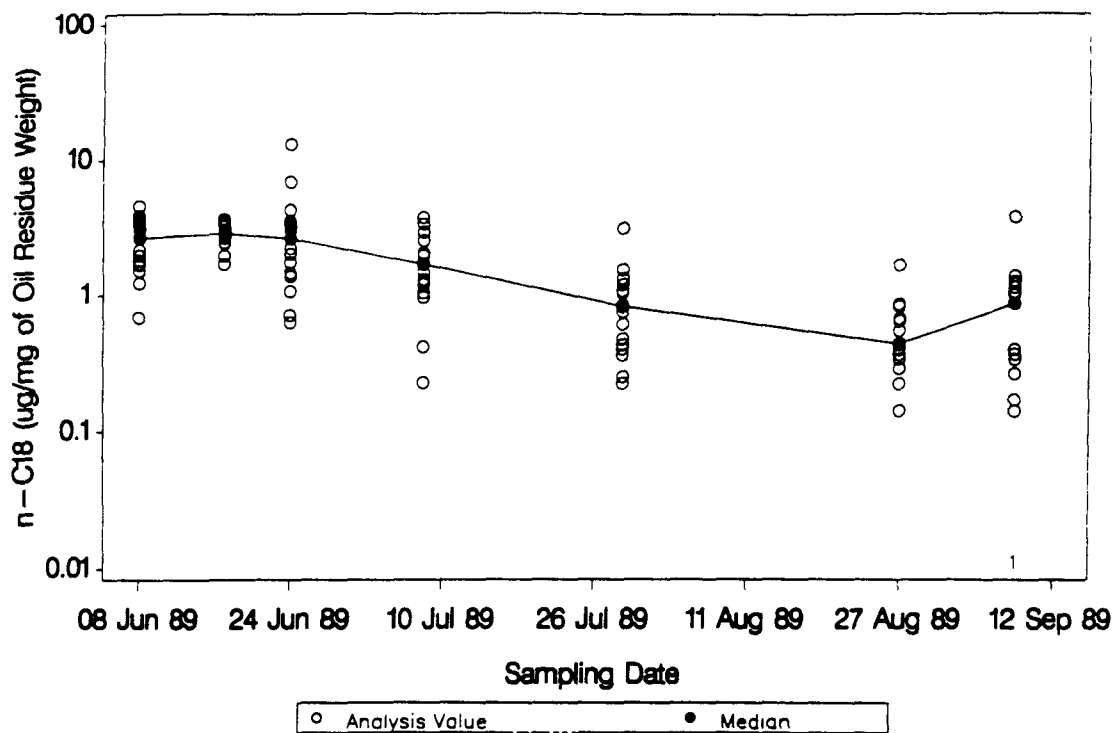
Figure 6.61. Change in nC18 Concentration Through Time for Eagle Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
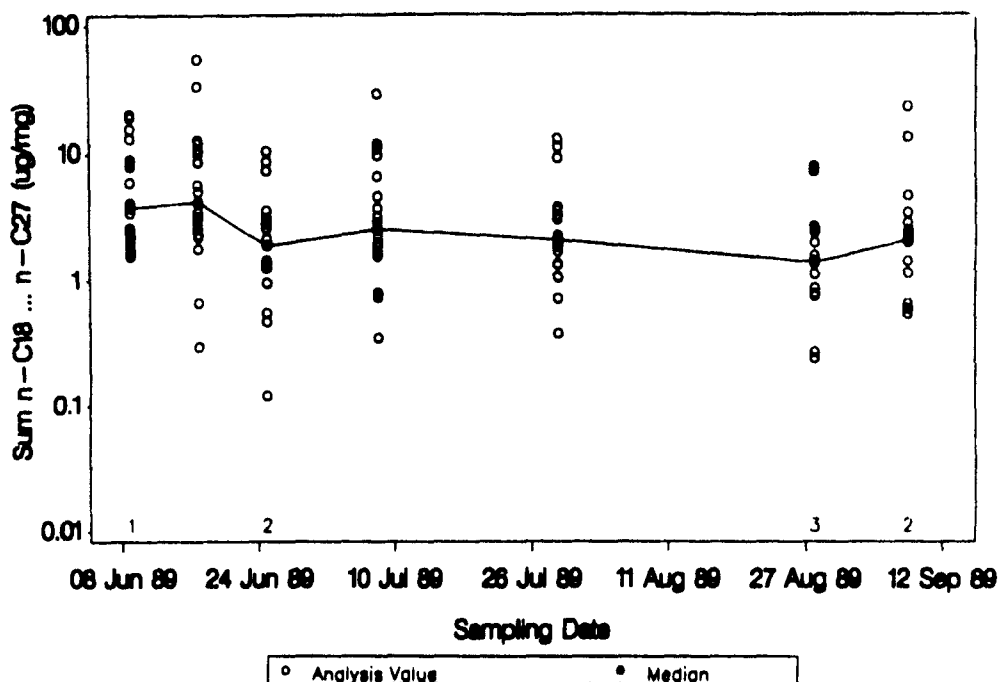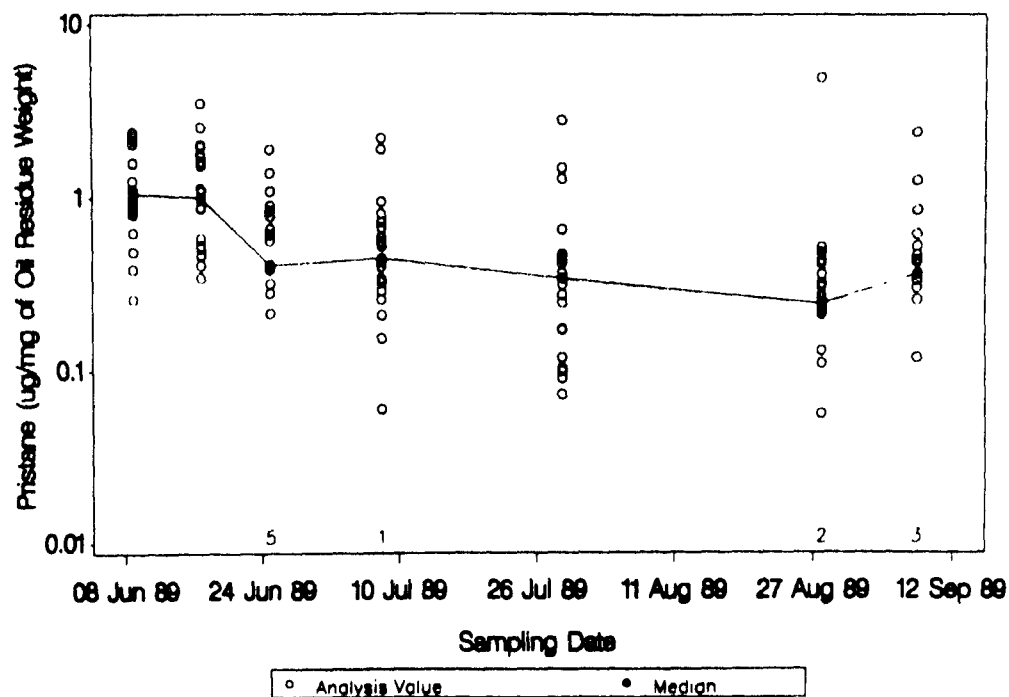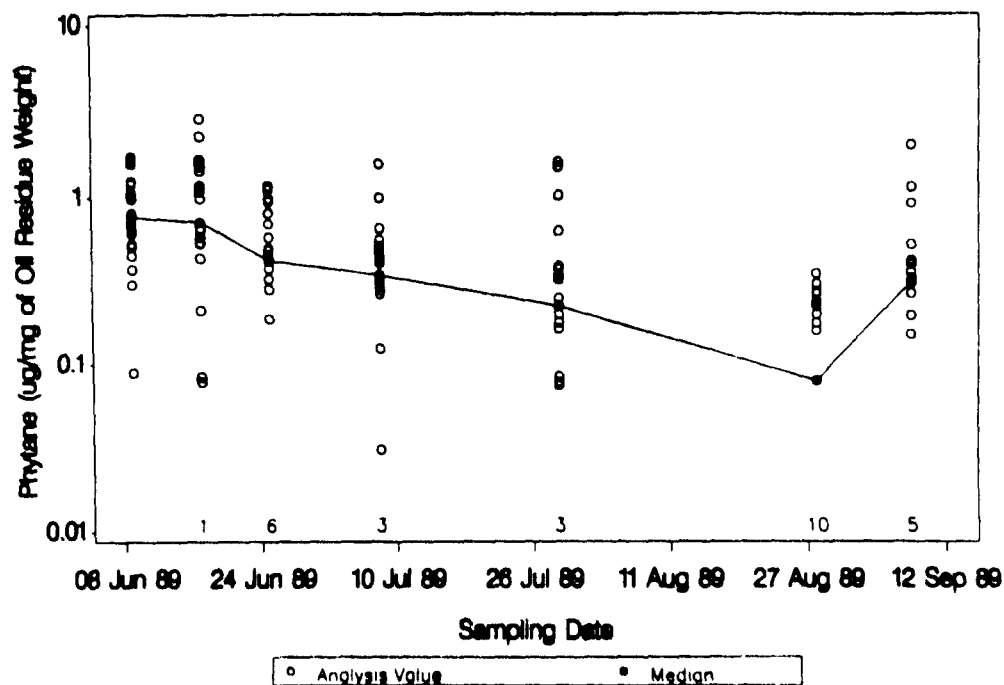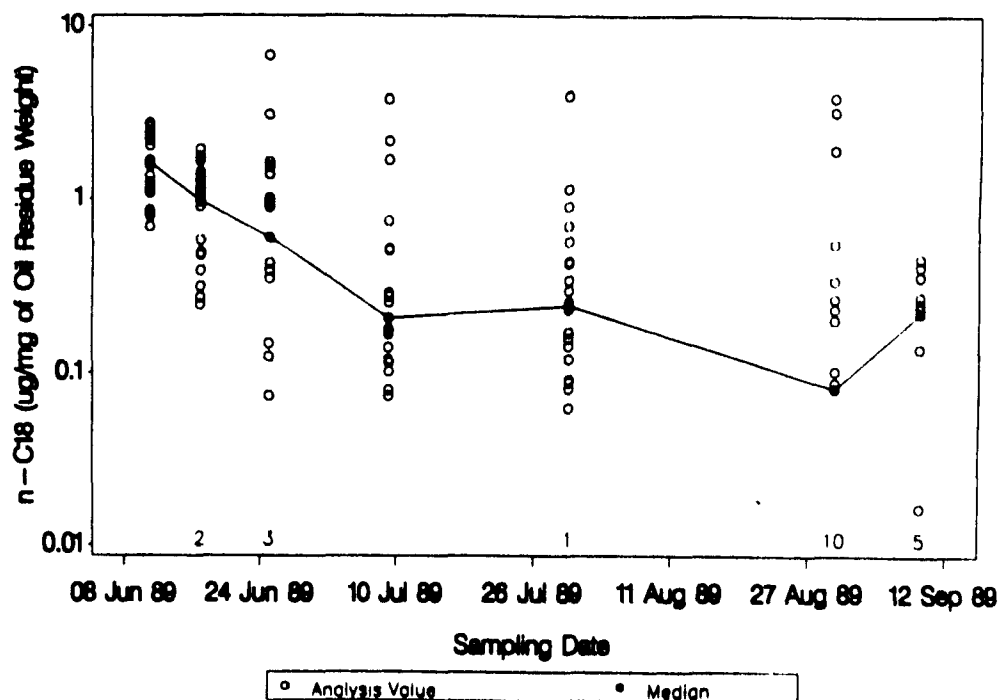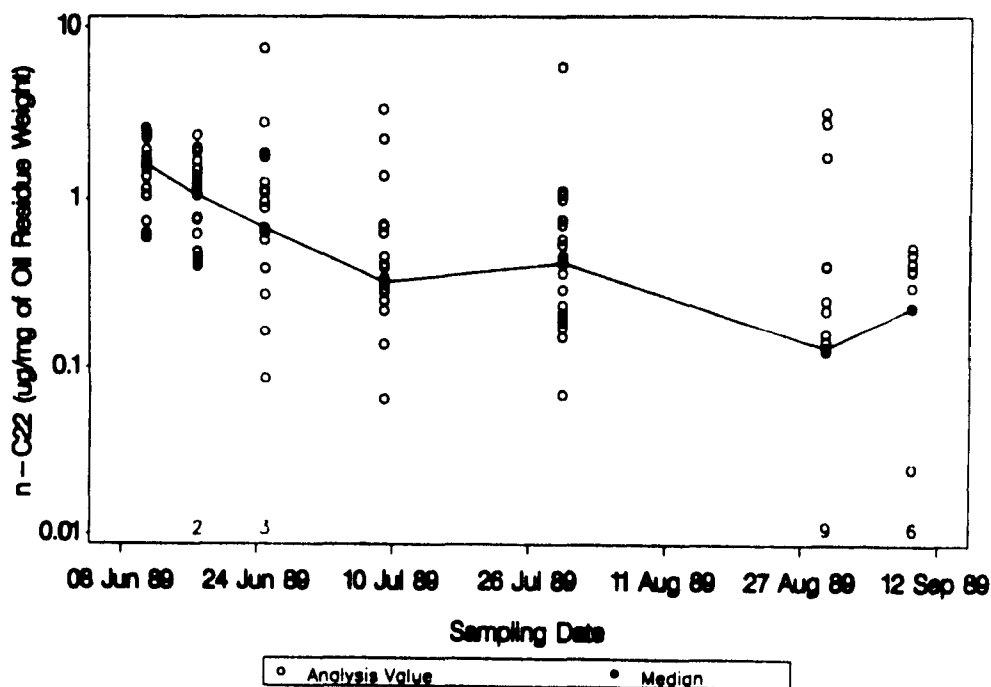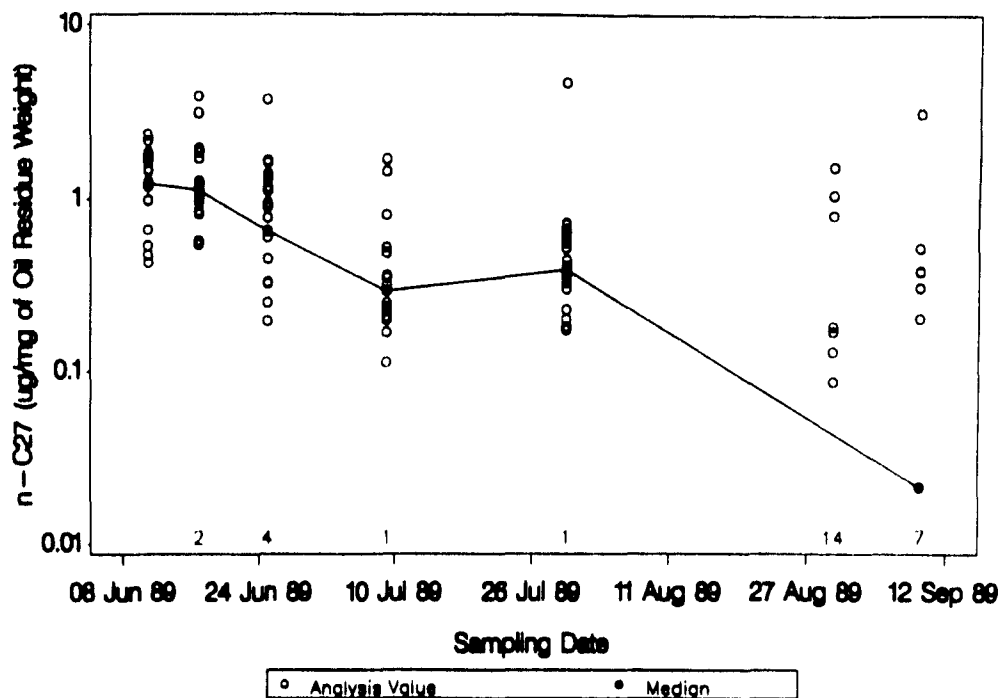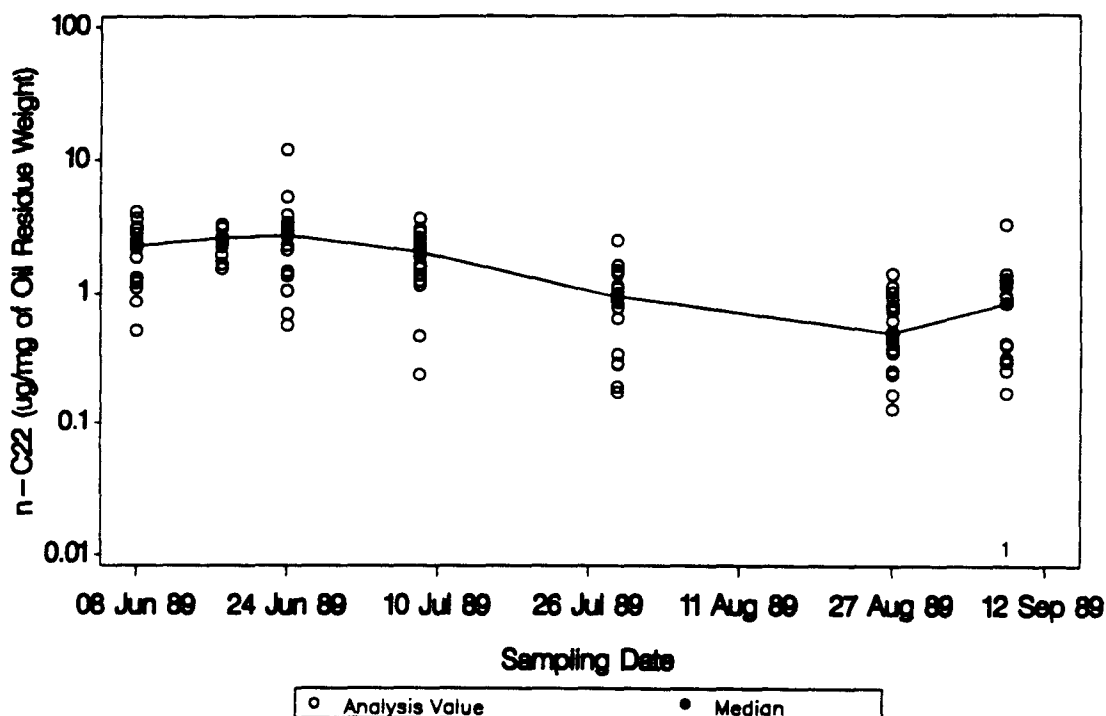

Figure 6.62. Change in nC22 Concentration Through Time for Eagle Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
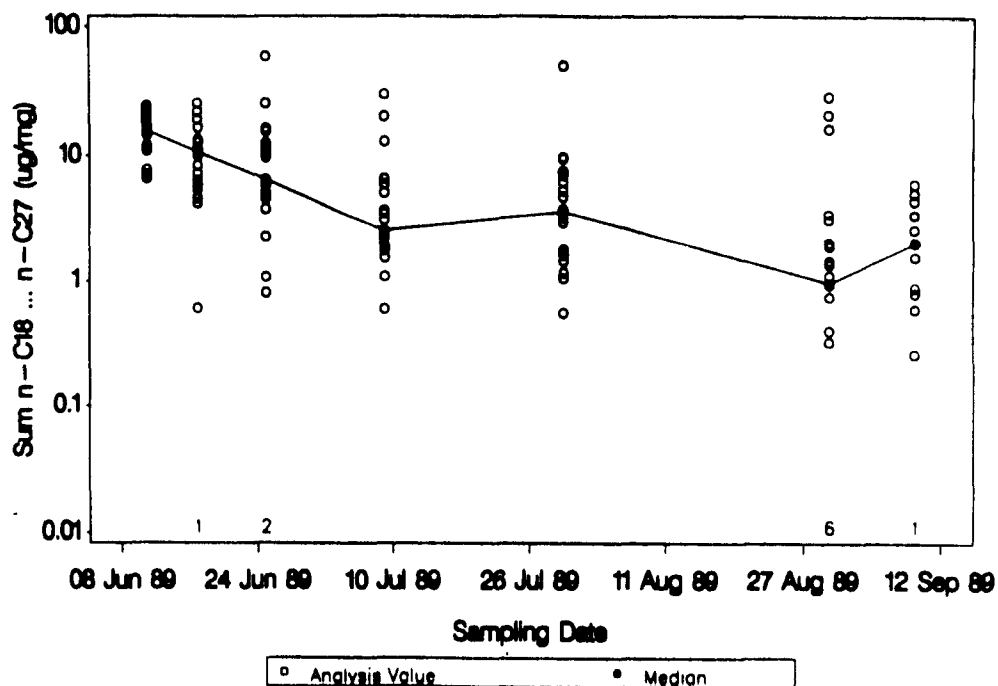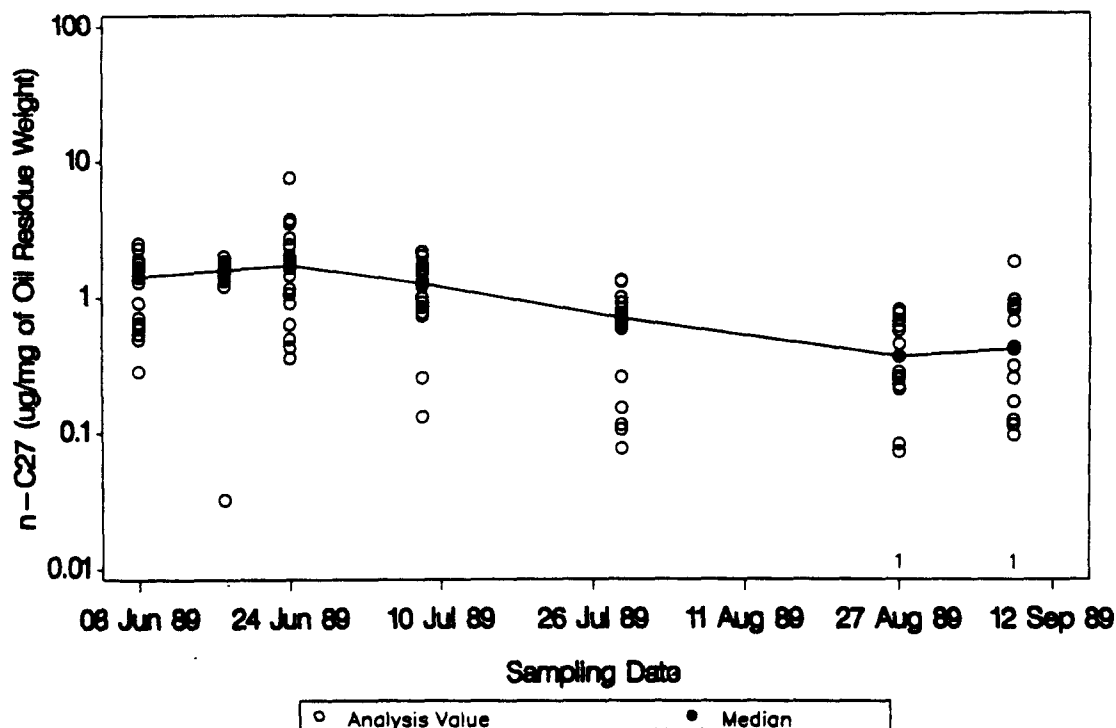
Figure 6.63. Change In nC27 Concentration Through Time for Eagle Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.
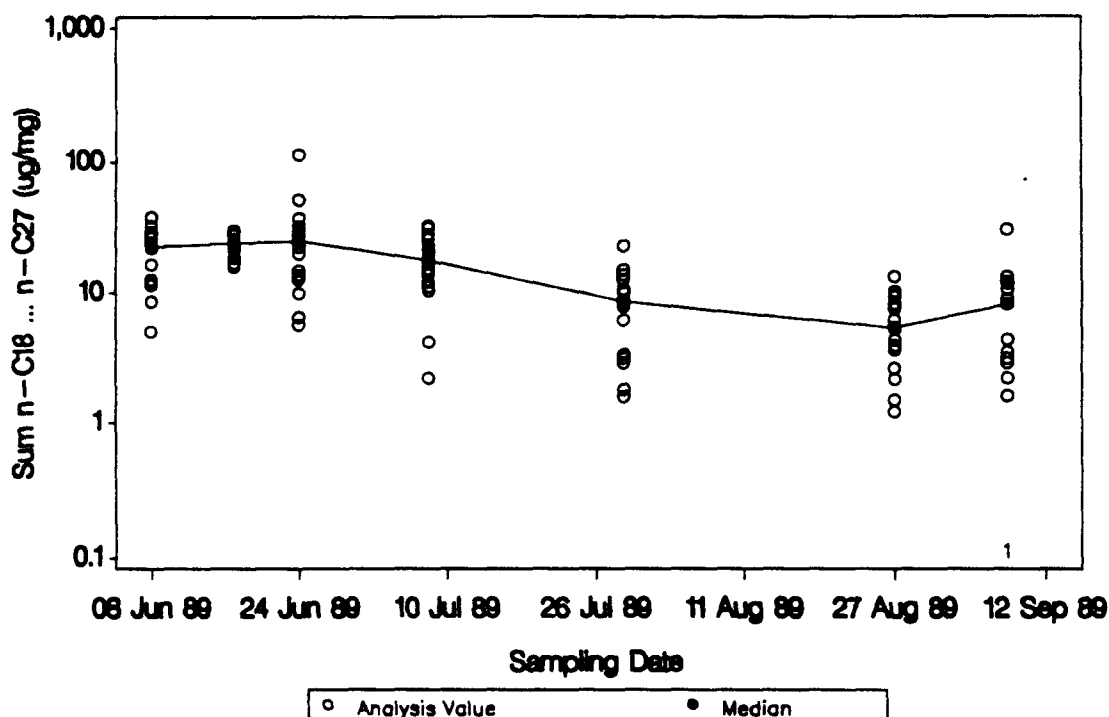

Figure 6.64. Change In Sum of the Alkane Concentration nC18 to nC27 Through Time for Eagle Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.
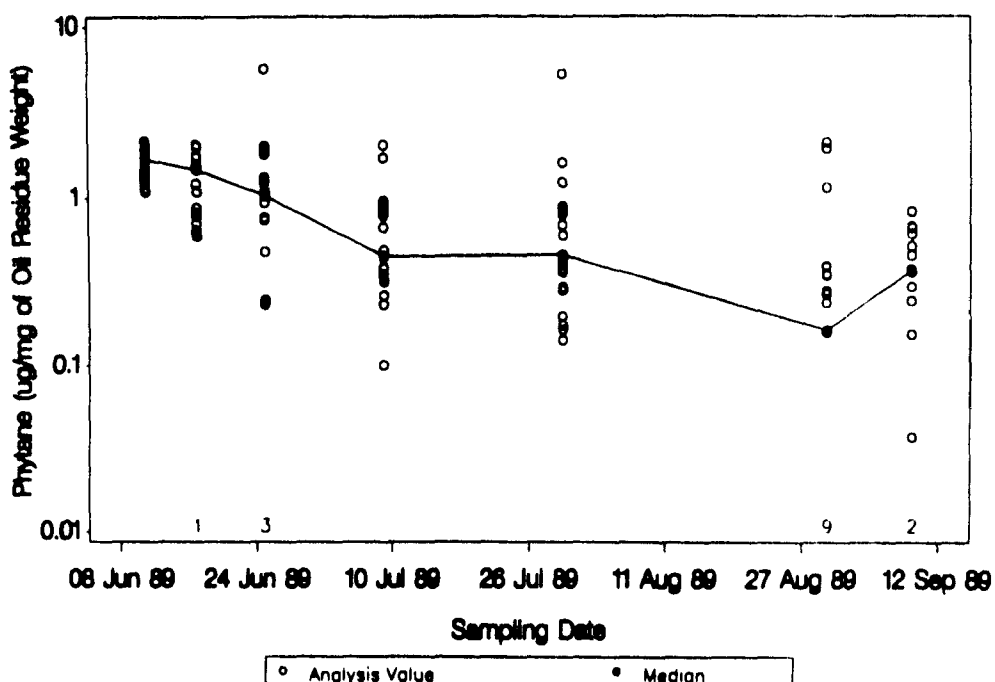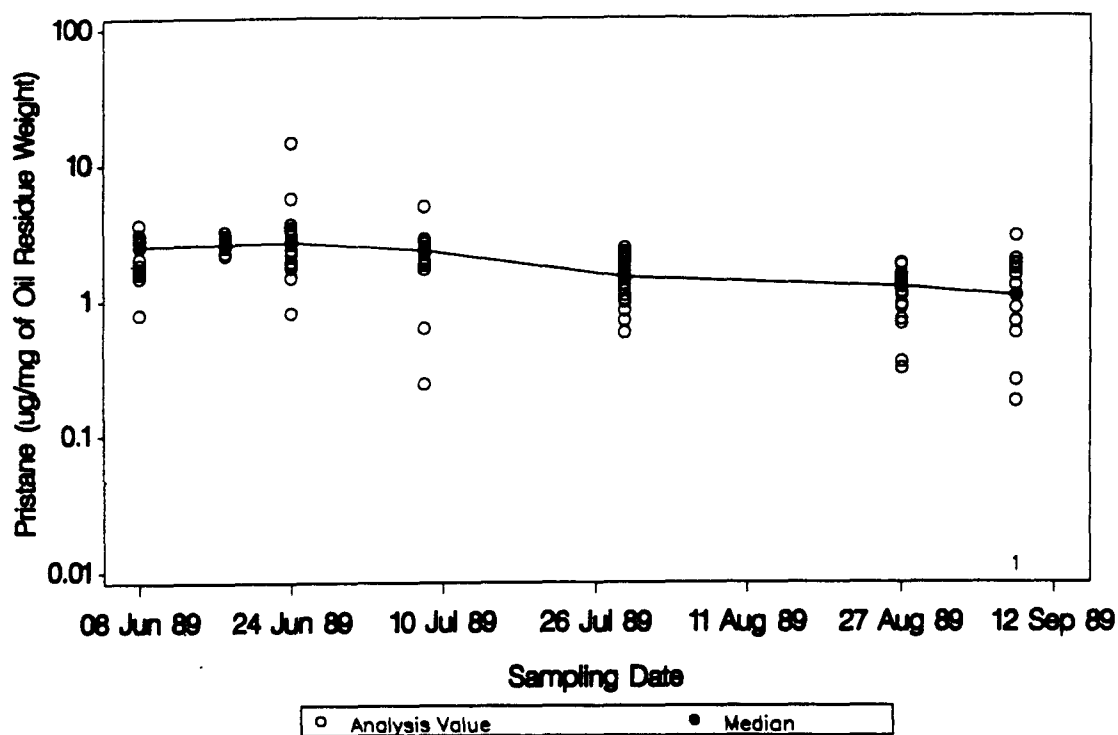
Figure 6.65. Change in Pristane Concentration Through Time for Eagle Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
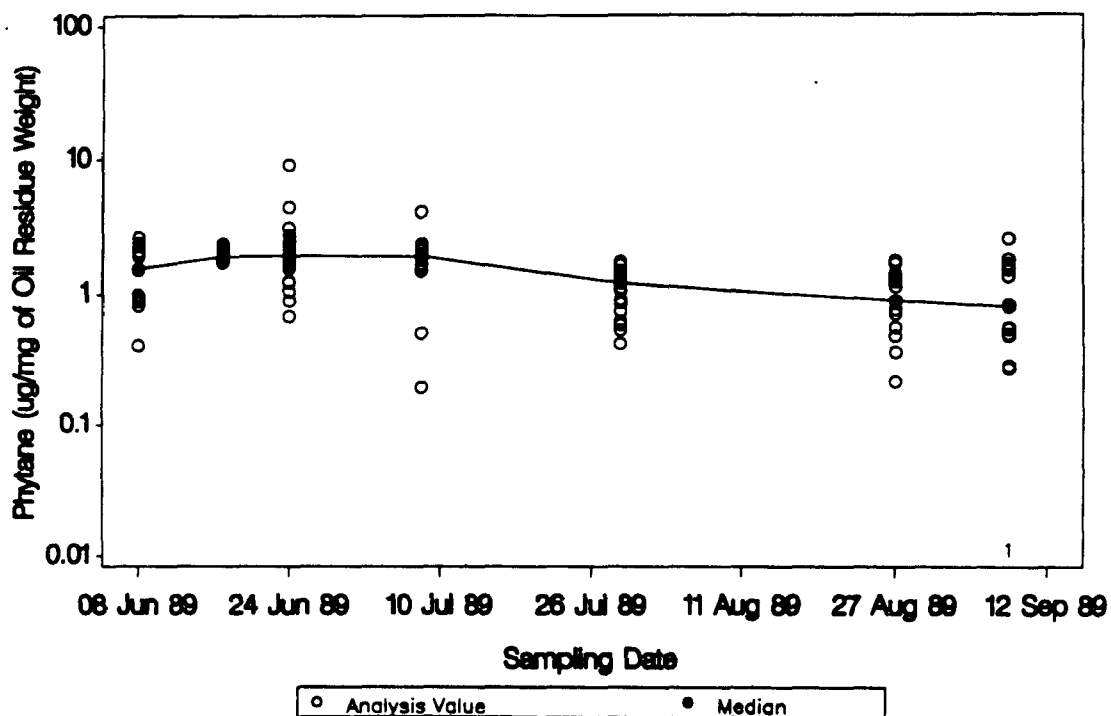
Figure 6.66. Change in Phytane Concentration Through Time for Eagle Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
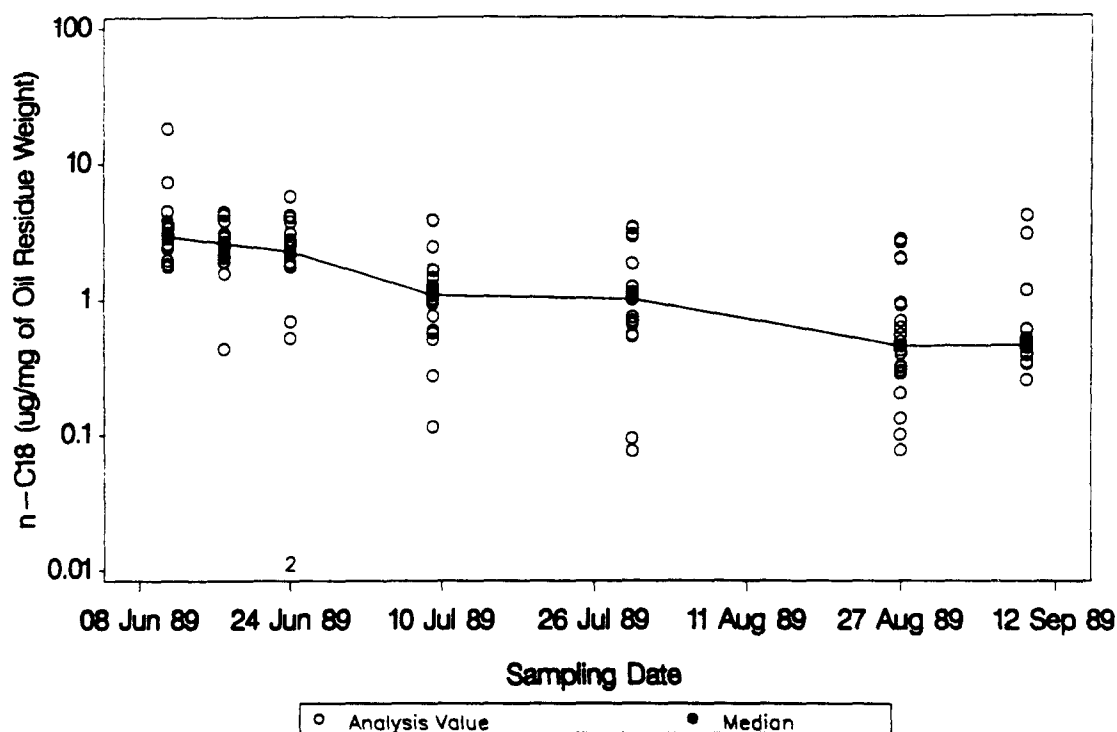
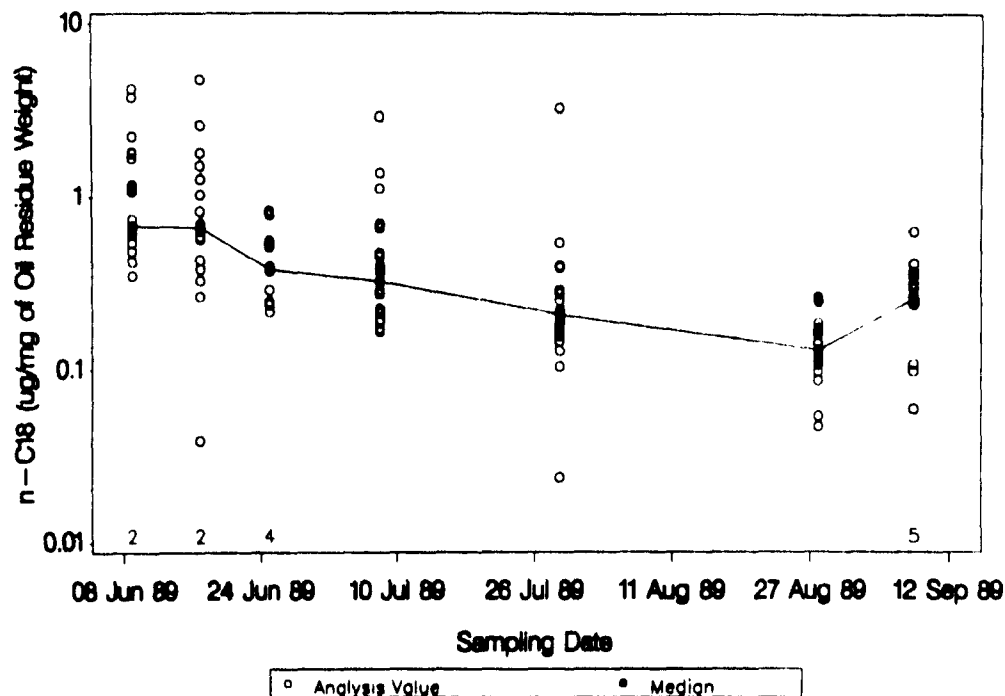**Figure 6.67. Change in nC18 Concentration Through Time for Otter Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.**
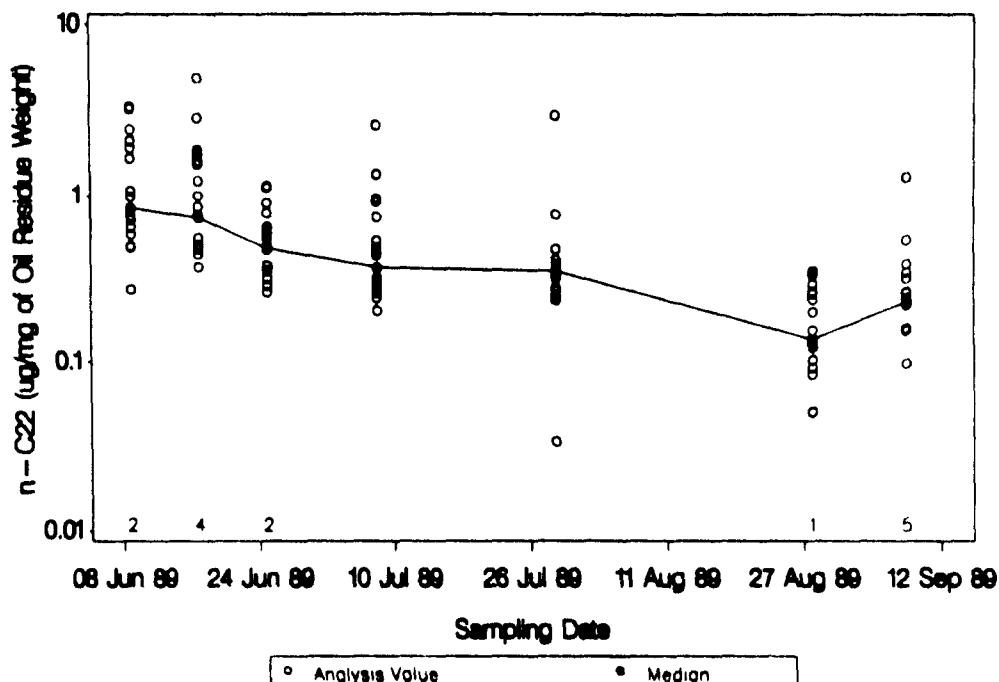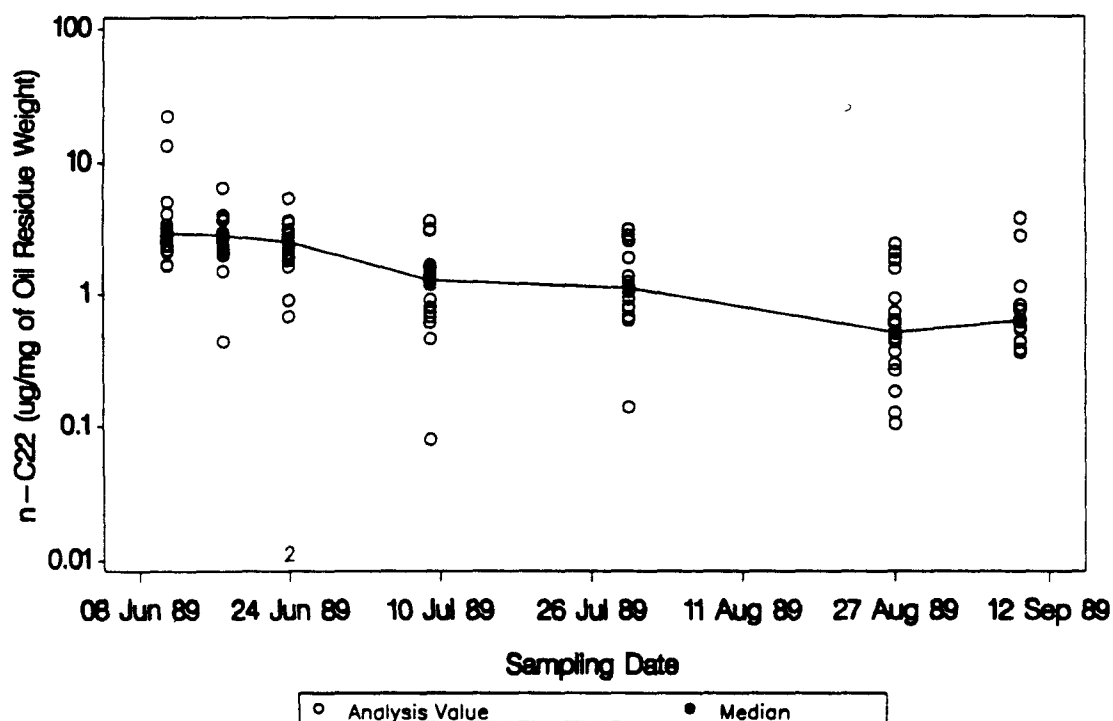

Figure 6.68. Change in nC22 Concentration Through Time for Otter Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
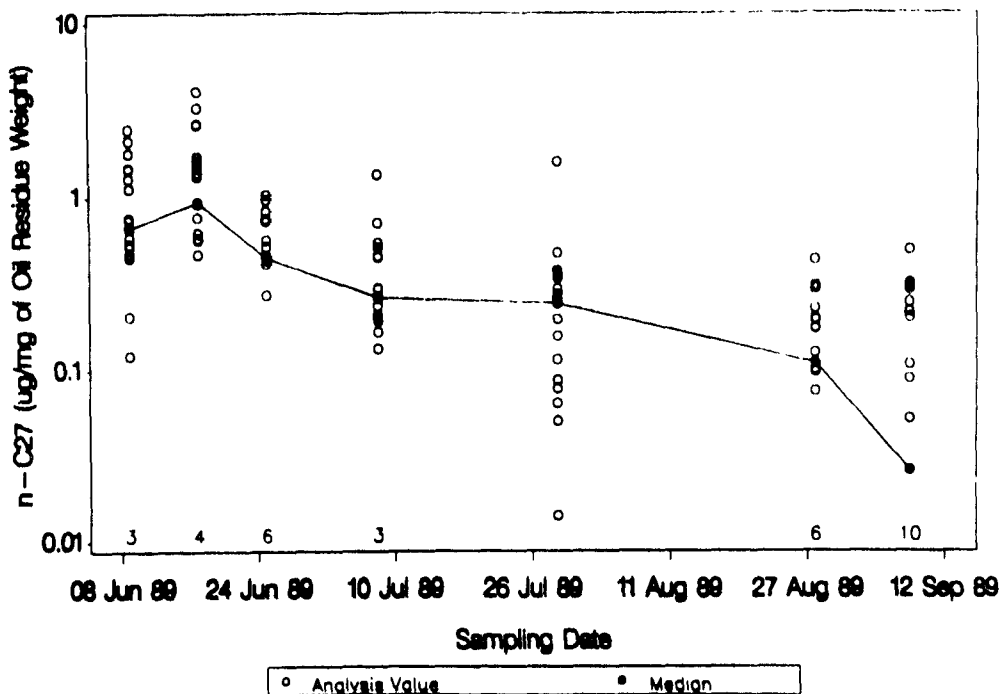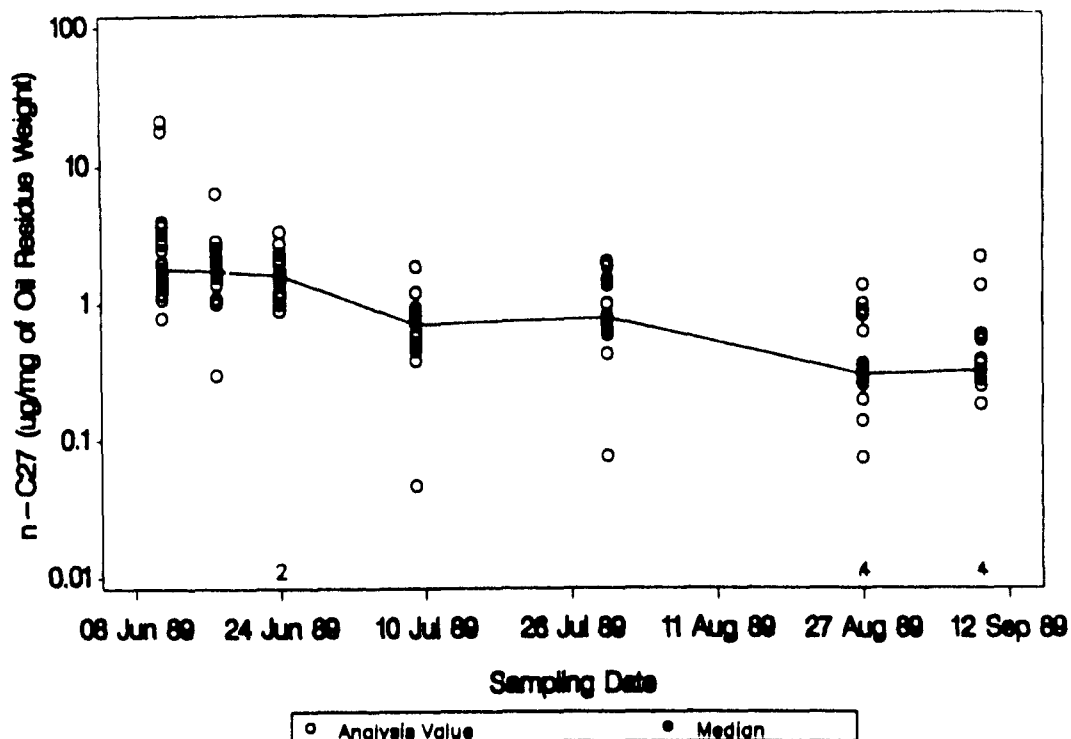
Figure 6.69. Change in nC27 Concentration Through Time for Otter Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
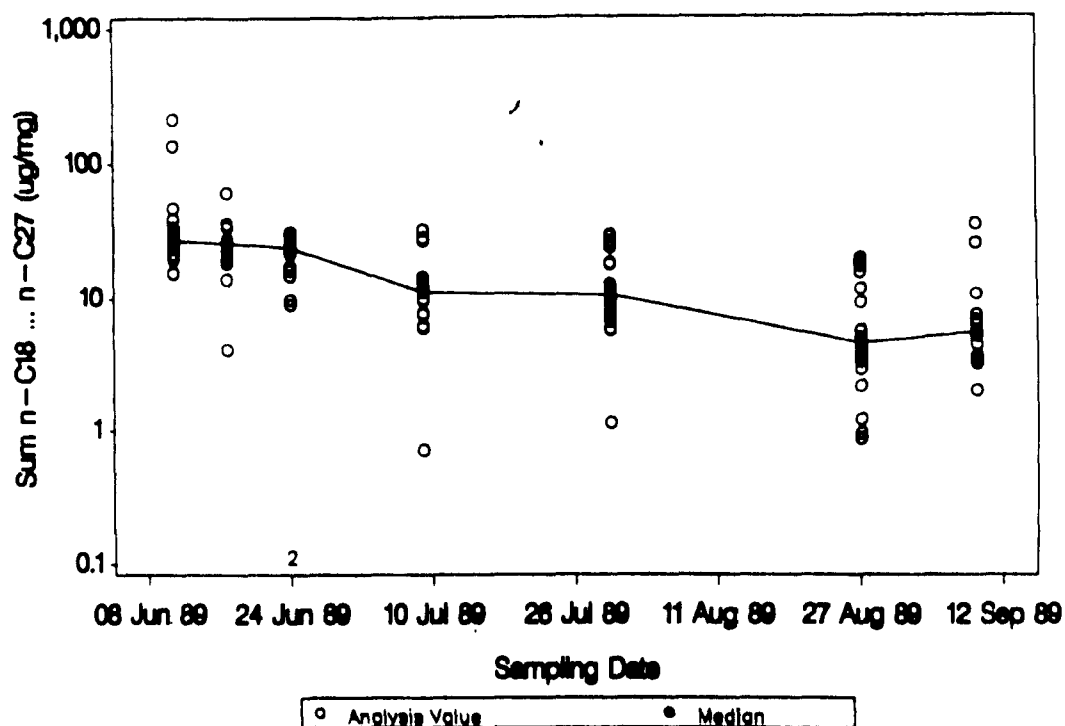
Figure 6.70. Change in Sum of Alkane Concentration nC18 to nC27 Through Time for Otter Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
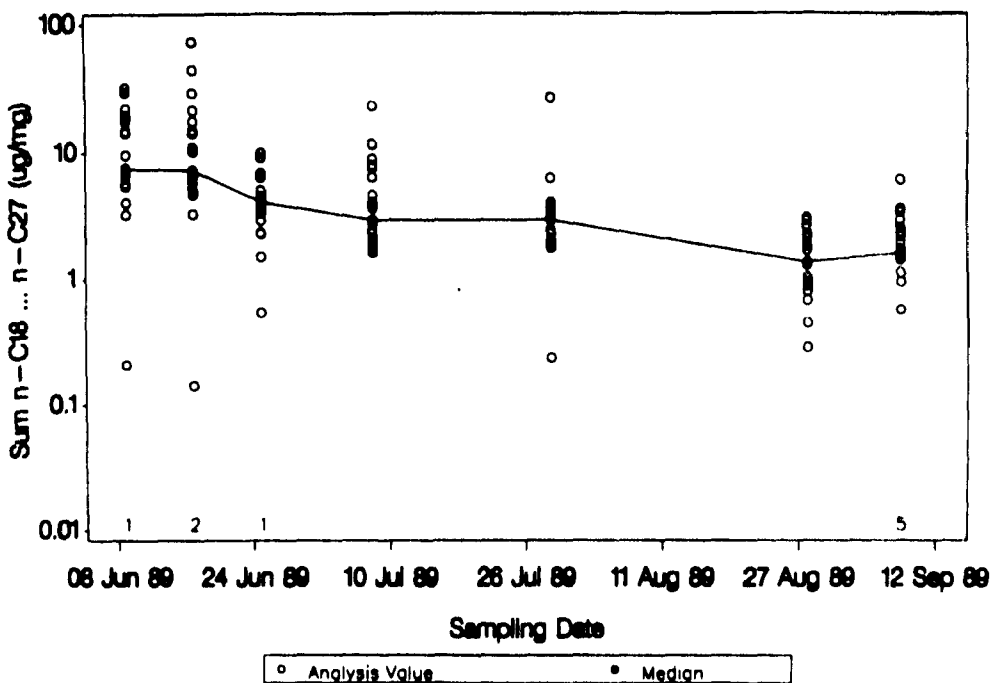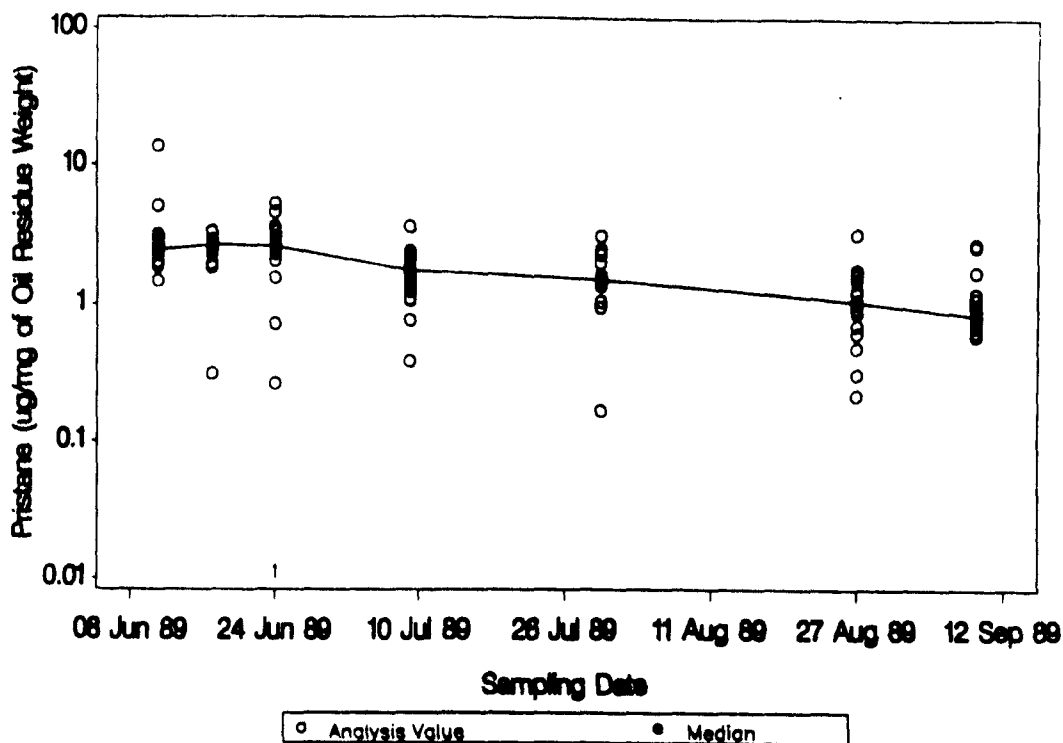
Figure 6.71. Change in Pristane Concentration Through Time for Otter Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
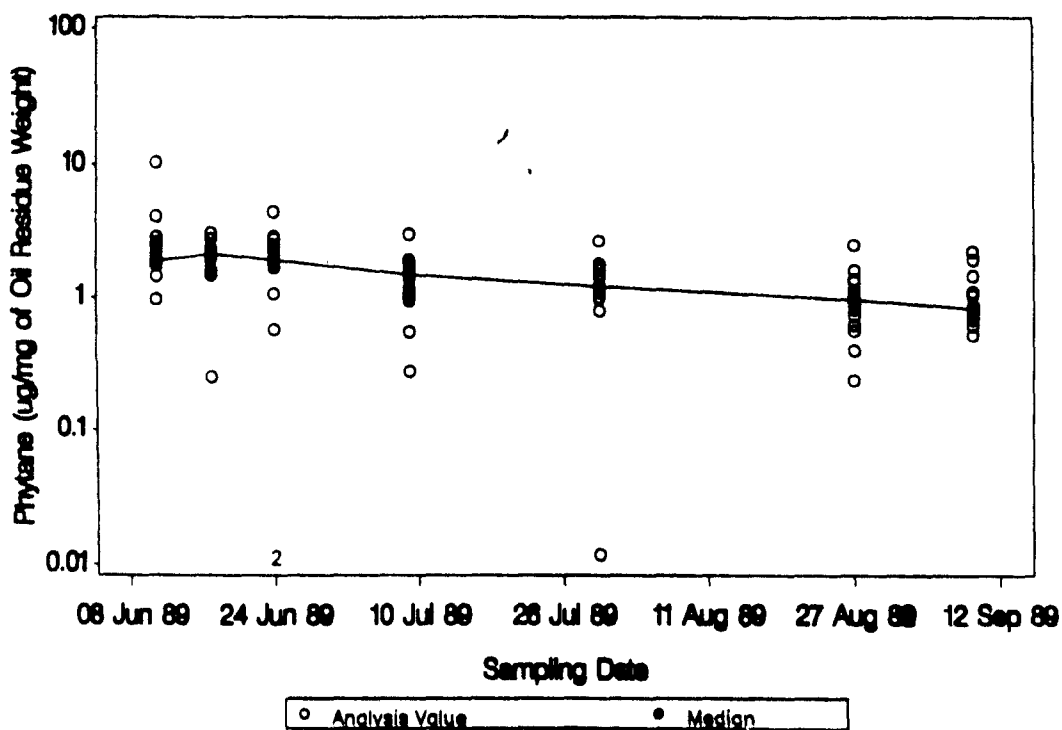
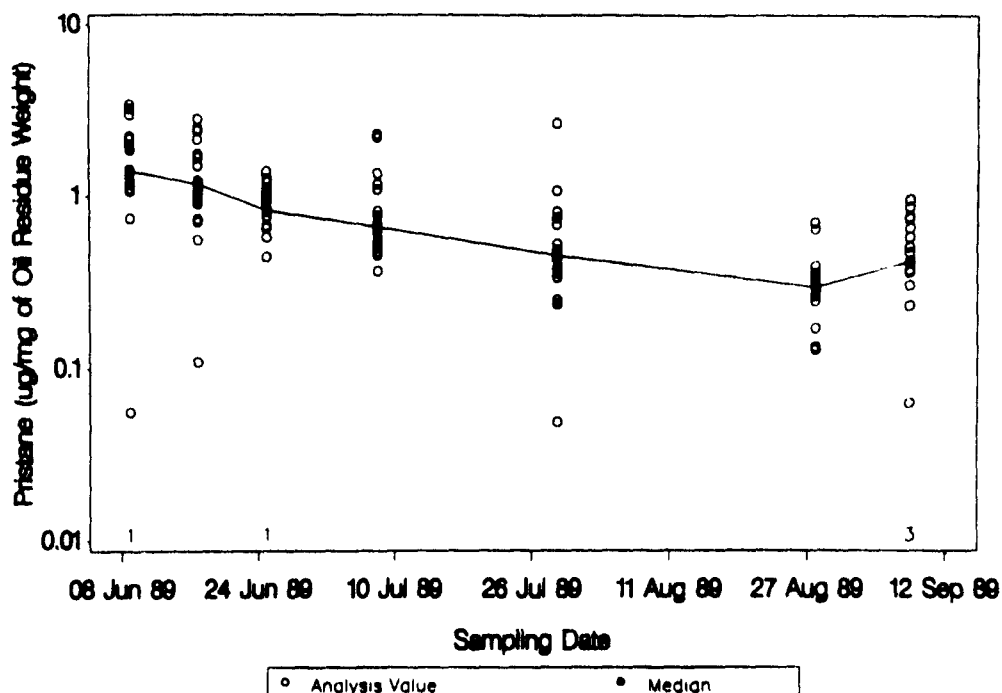**Figure 6.72.** Change in Phytane Concentration Through Time for Otter Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
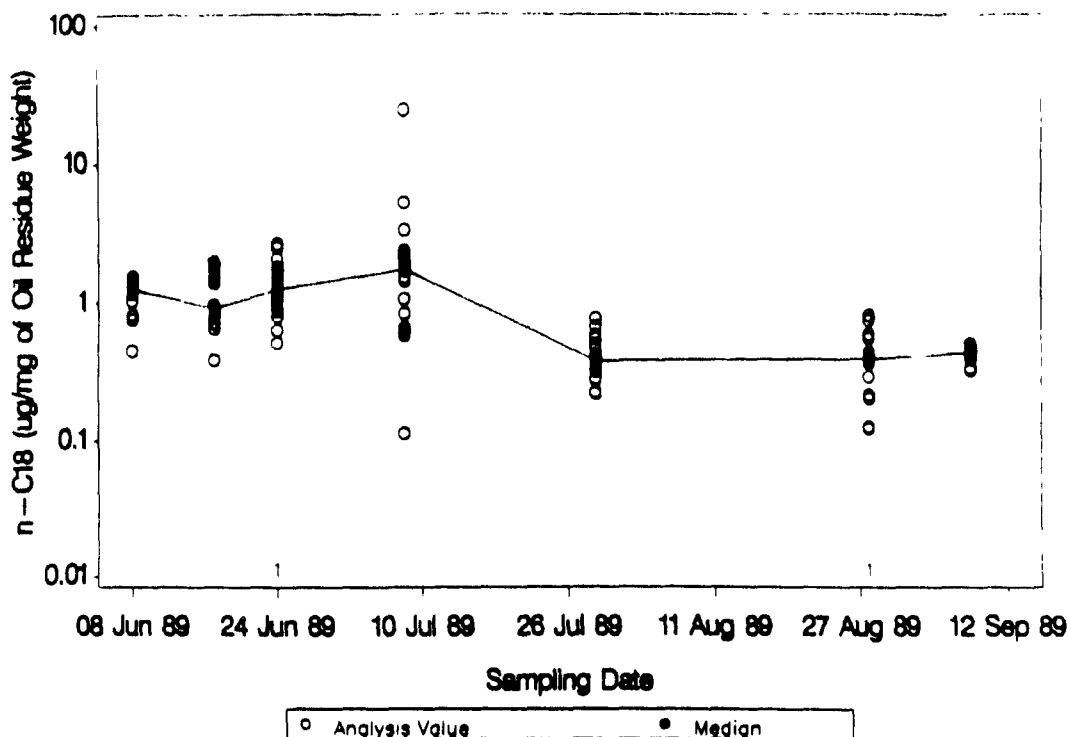
Figure 6.73. Change in nC18 Concentration Through Time for Otter Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
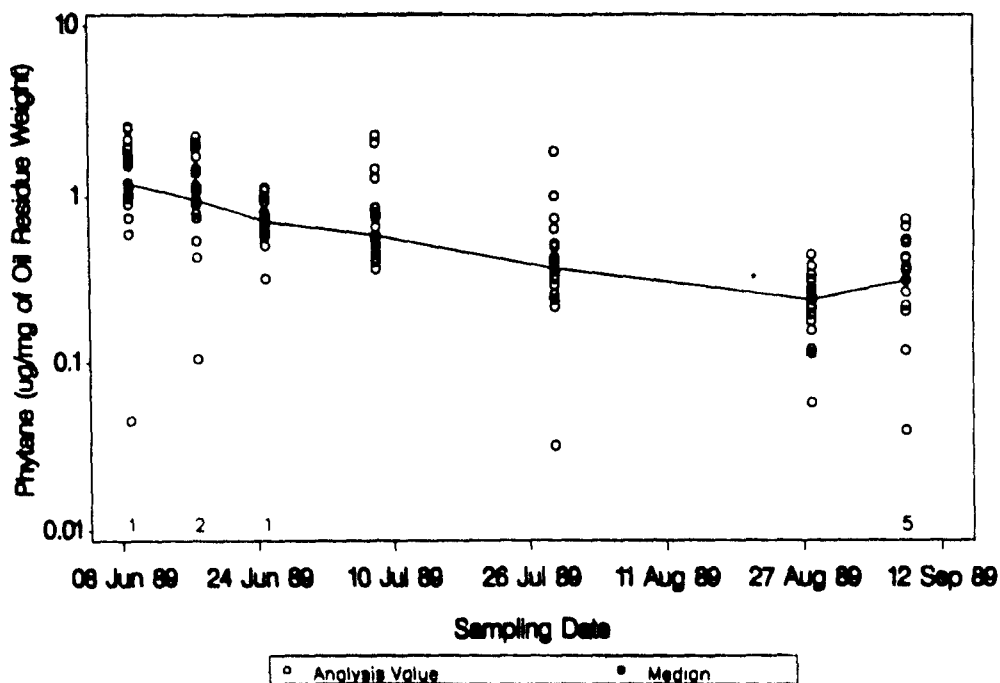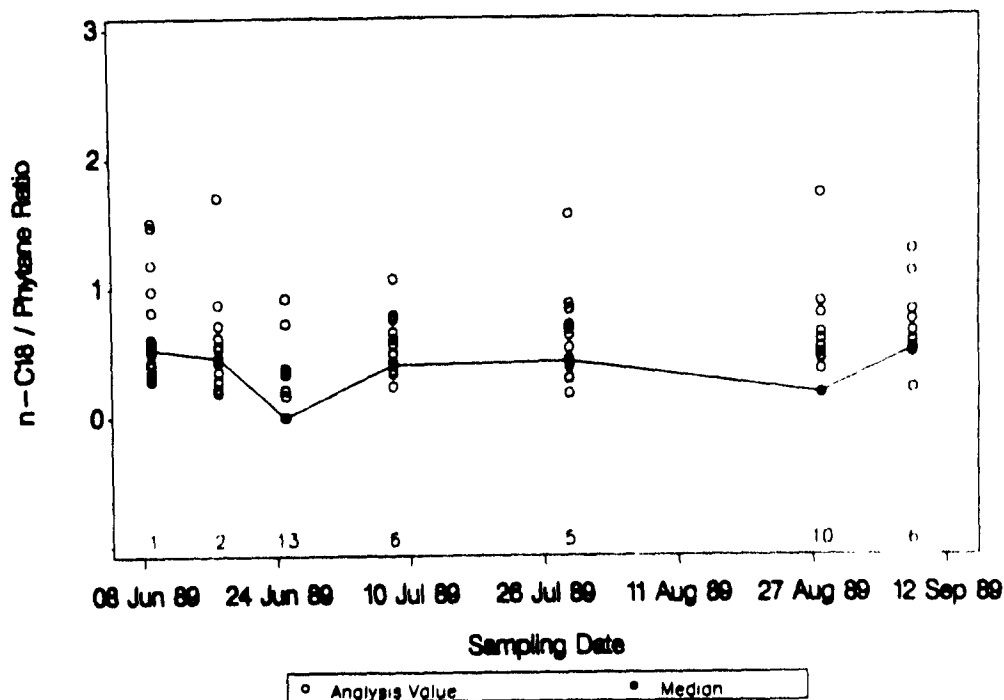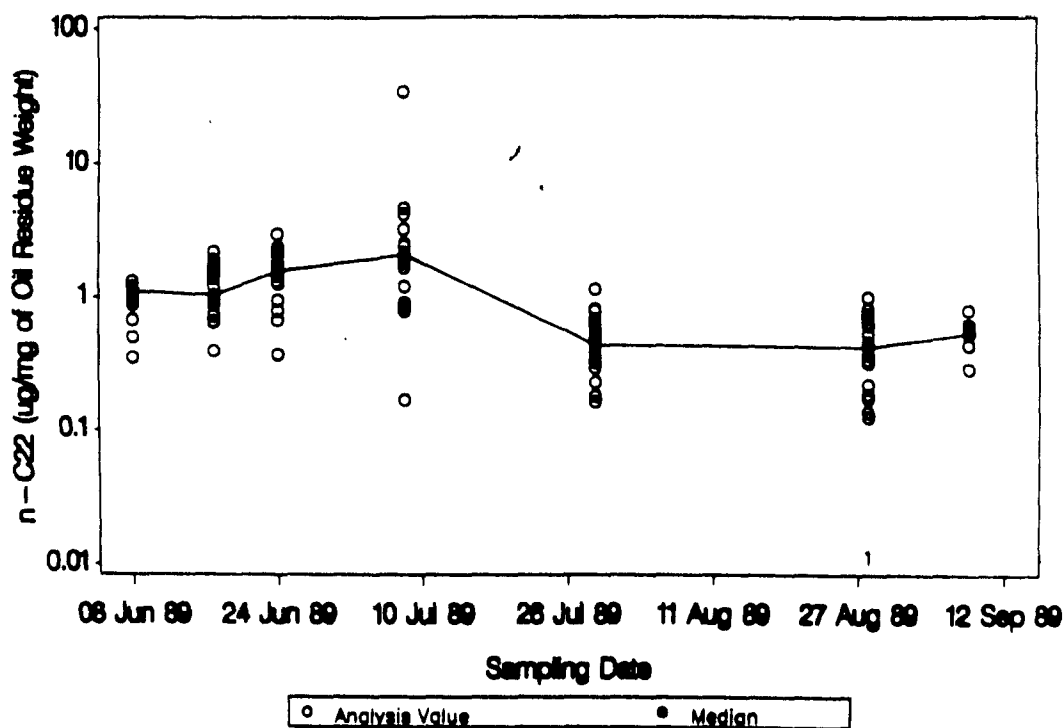

Figure 6.74. Change in nC22 Concentration Through Time for Otter Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
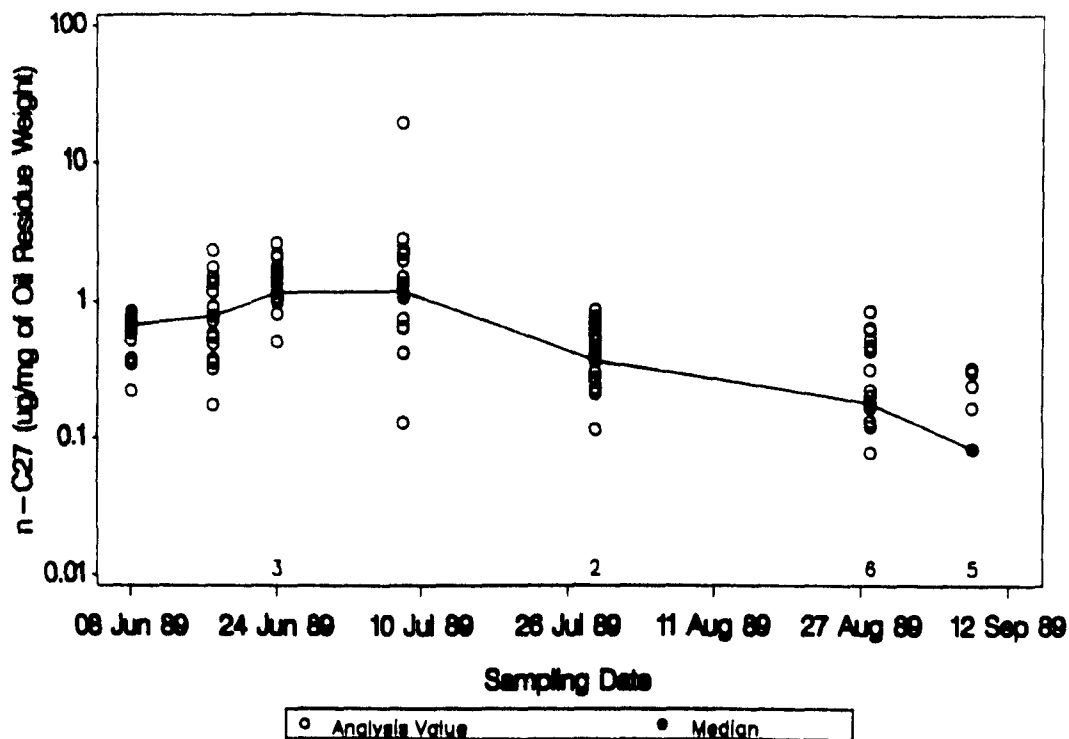
Figure 6.75. Change in nC27 Concentration Through Time for Otter Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
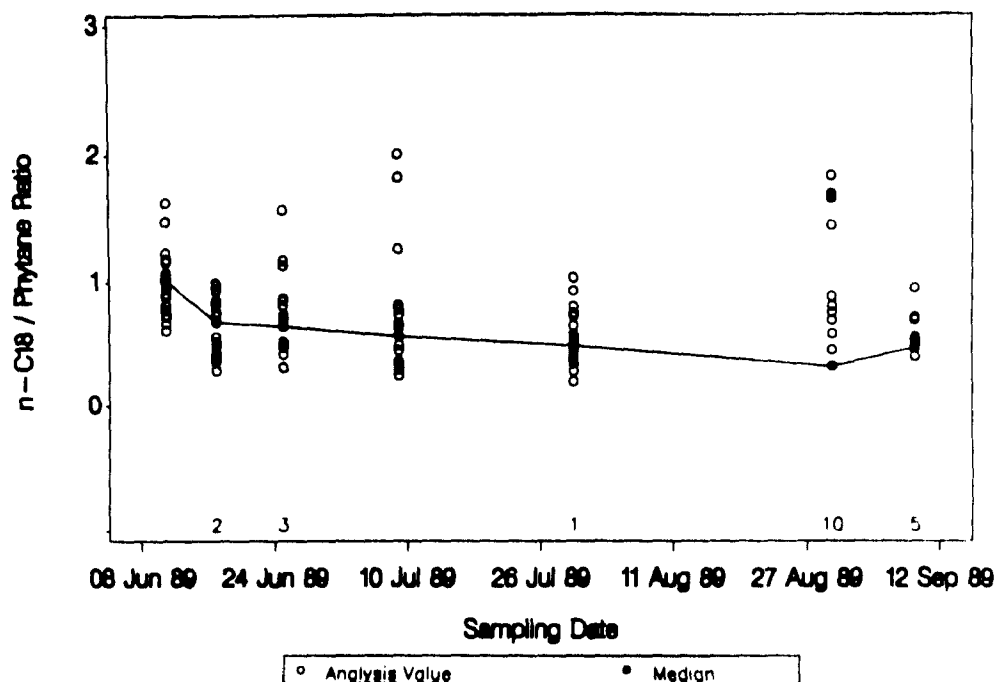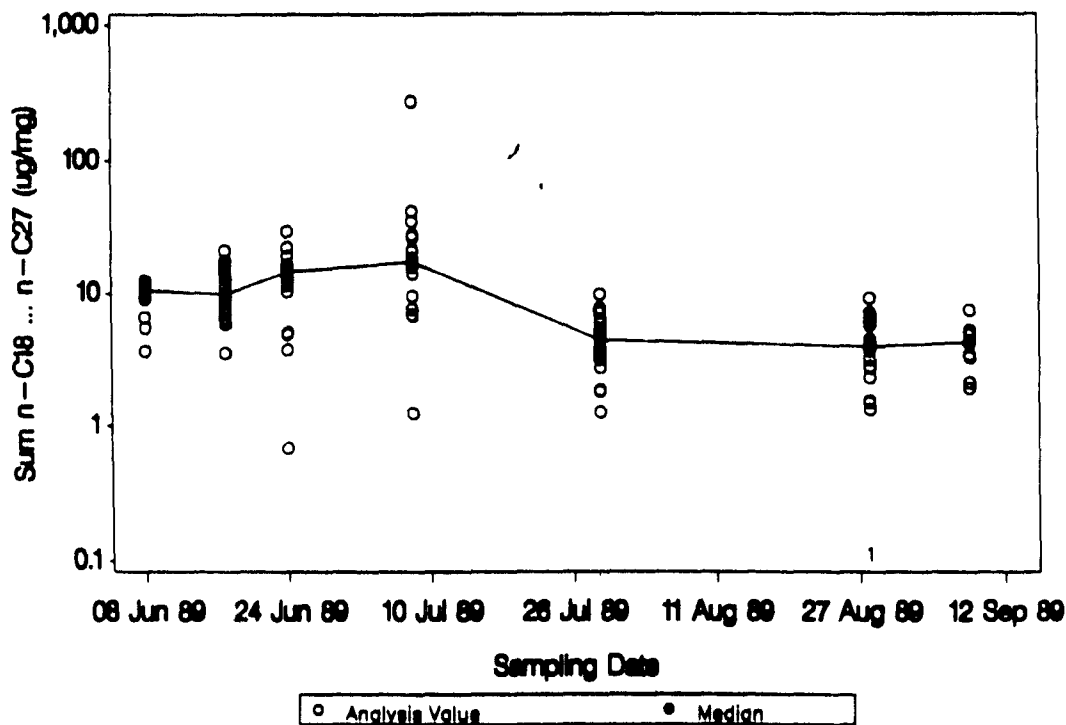

Figure 6.76. Change in Sum of the Alkane Concentration nC18 to nC27 Concentration Through Time for Otter Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
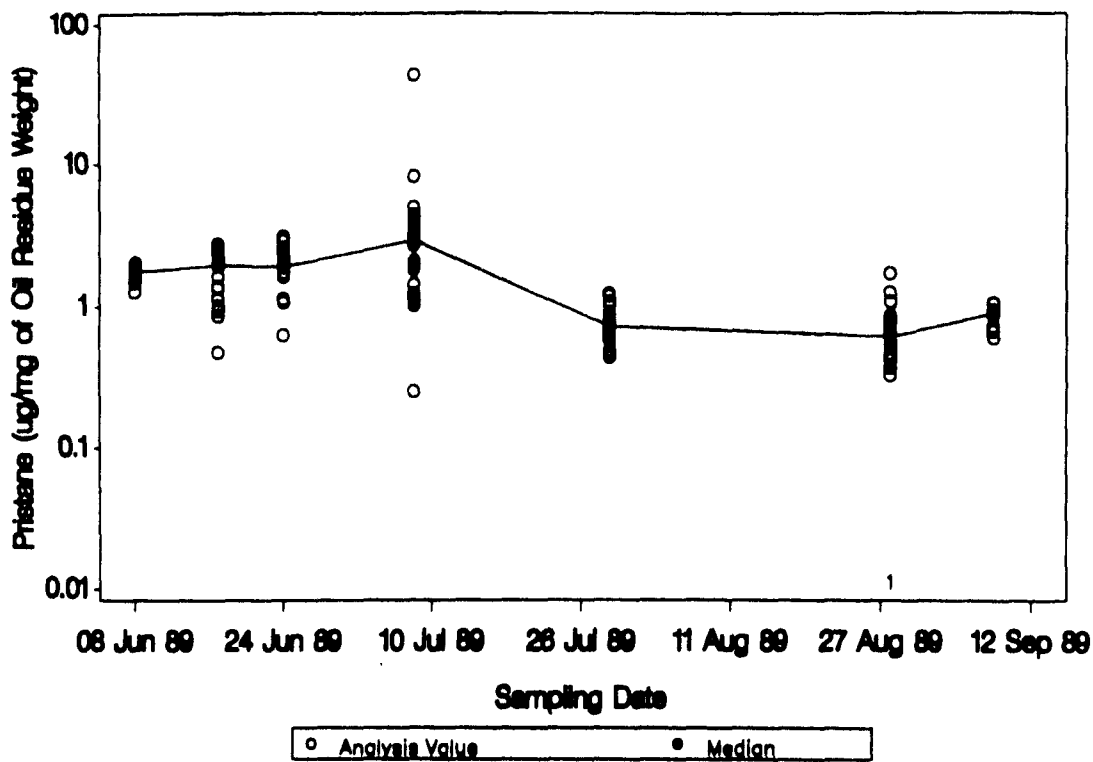
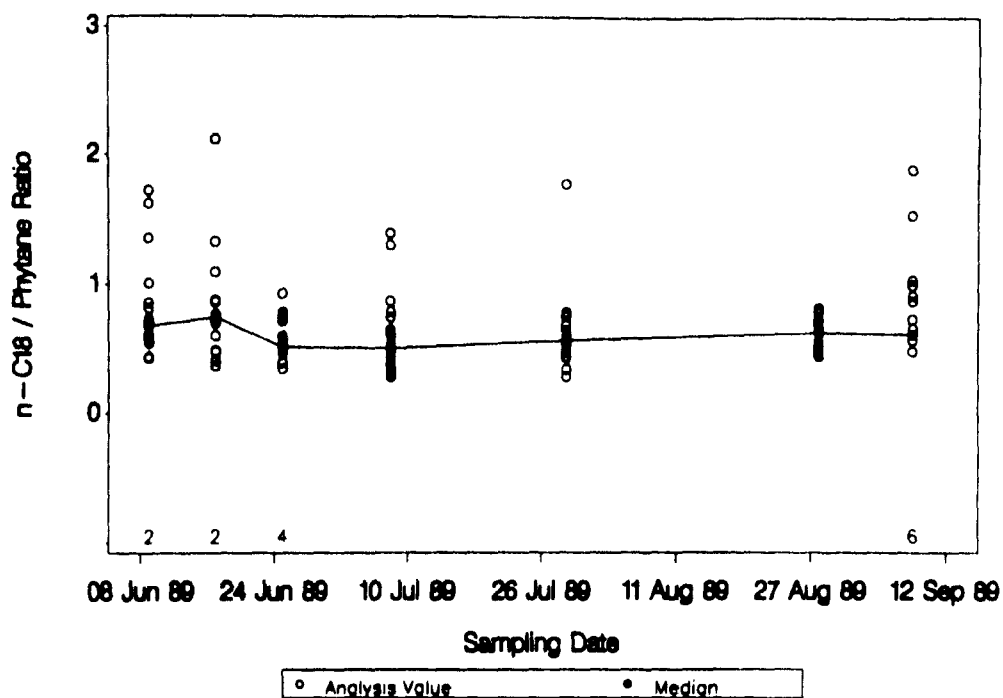**Figure 6.77.** Change in Pristane Concentration Through Time for Otter Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
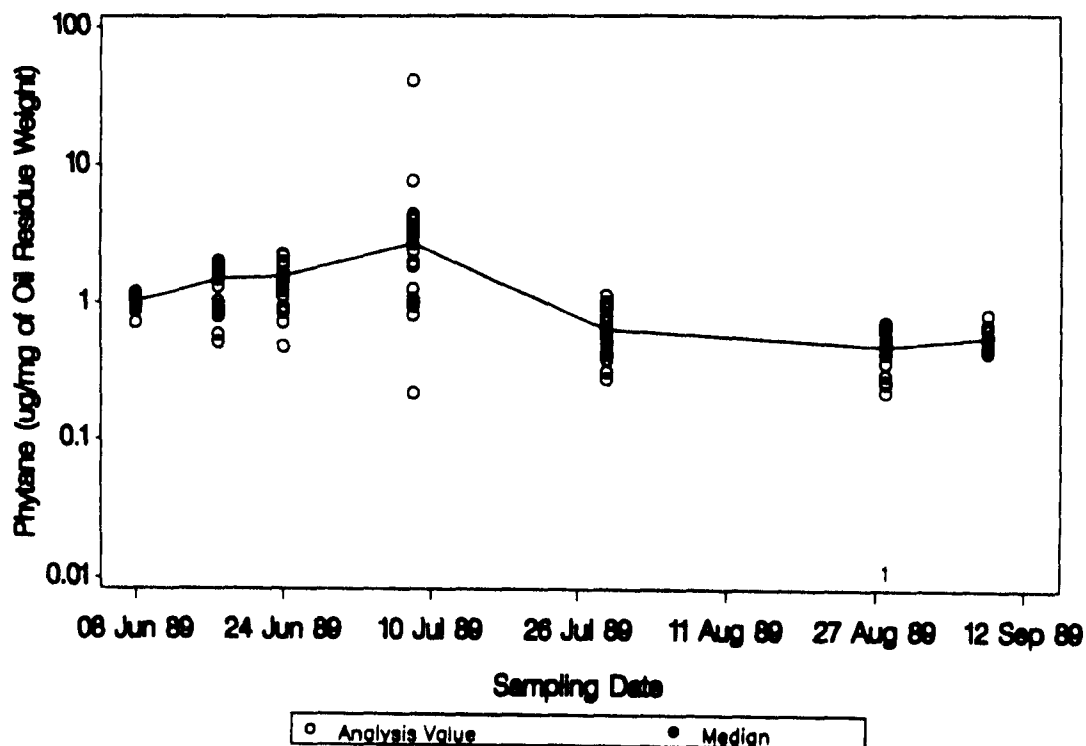
Figure 6.78. Change in Phytane Concentration Through Time for Otter Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
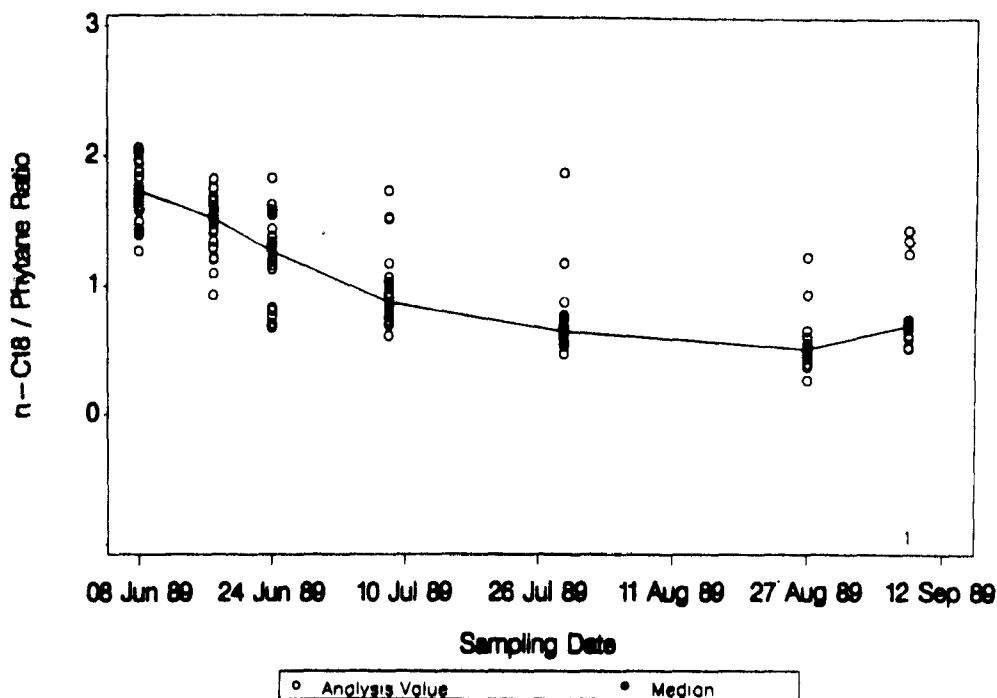
Figure 6.79. Change in nC18/phytane Ratio Through Time for Eagle Beach (Untreated Control) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
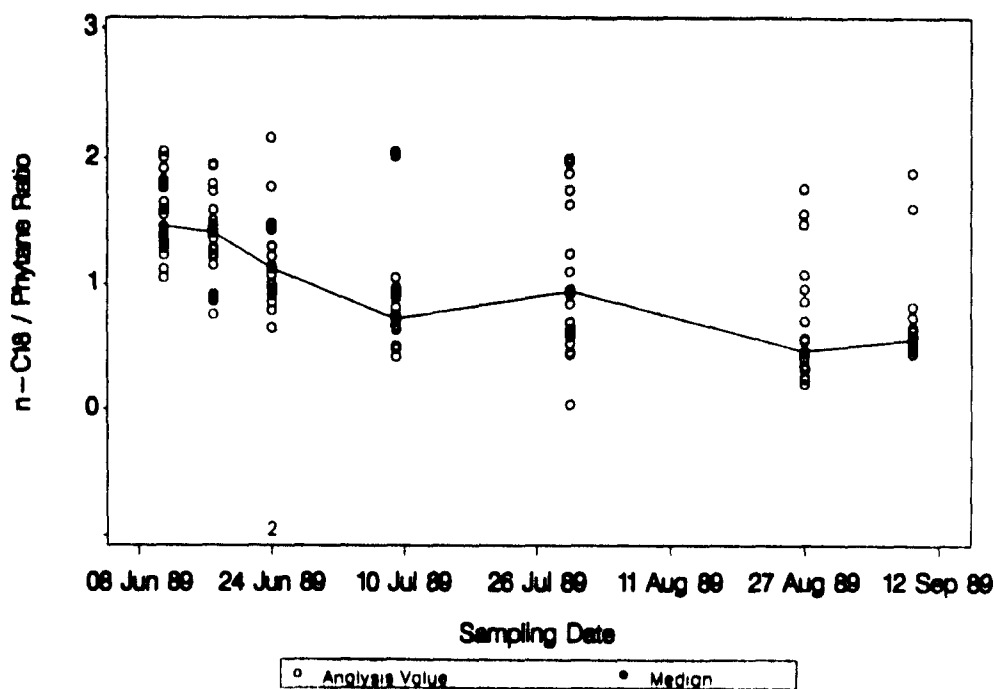
Figure 6.80. Change in nC18/Phytane Ratio Through Time for Otter Beach (WOODACE Briquettes) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
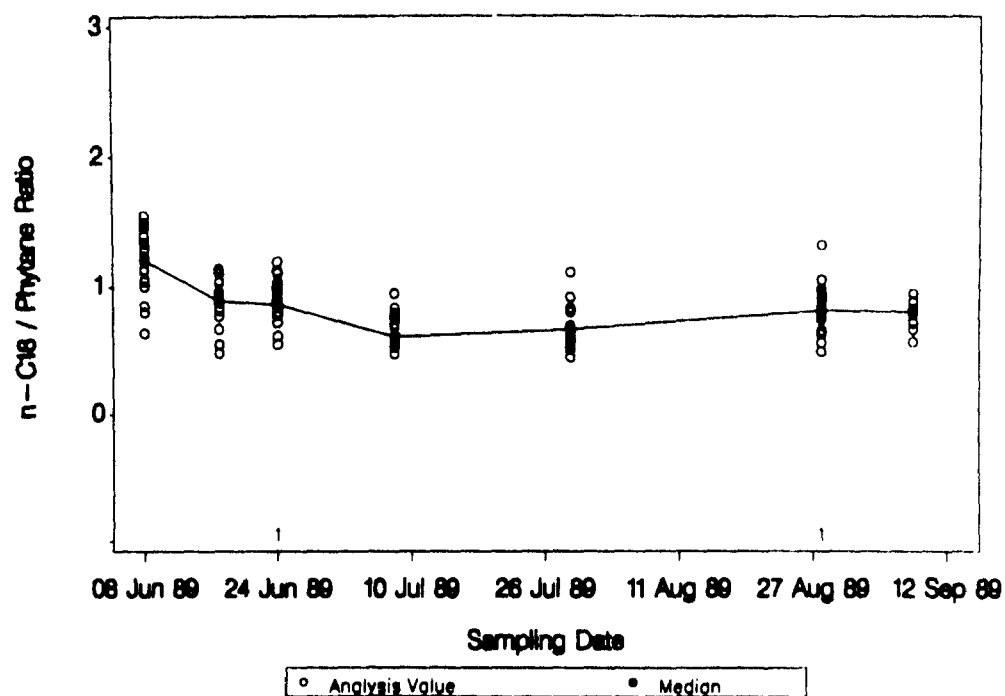
Figure 6.81. Change in nC18/phytane Ratio Through Time for Otter Beach (INIPOL) at Snug Harbor (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

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Mann-Whitney test, hydrocarbon concentrations in samples from these two beaches were not significantly different at the 95% confidence level at the t=0 sample (Table 6.19). Figure 6.82 also provides a graphic representation of the percent change in the medians. However, hydrocarbon concentrations were significantly different on the July 8 sampling date; the median values on the treated plot had decreased by approximately 50-60%, but on the untreated control plot they had decreased only 20-25%. Therefore, the decay rate for the summed alkanes on the briquette fertilizer-treated plot was significantly different from zero over this initial 29 day period but the decay rate on the untreated control plot was not.

By the following sampling date (day 4, July 29), the untreated control appeared to have "caught up" with the briquette fertilizer-treated plot to some extent; i.e., no differences between the concentrations of the alkanes. This may have been due to a more accelerated decay on the untreated control and an apparent slower decay rate on the treated plot. However, over the entire duration of the study, it is clear that final concentrations of individual hydrocarbon concentrations on the briquette fertilizer-treated plots were considerably lower than on the untreated control. Decay rates overall differed by a factor of two for the summed alkanes, suggesting a significant long-term effect of this fertilizer application.

These changes in hydrocarbon composition can also be attributed to biodegradation since there was a significant decay in the nC18/phytane ratios on both plots. Differences between the plots were not significant, but this may be the result of a slightly greater decay rate for phytane on the briquette fertilizer-treated plot. Again, the ratio method may give a conservative indication of biodegradation if phytane is being degraded.

Finally, it would appear that the changes in oil composition were not directly coupled to losses in oil residue weight. For example, on the briquette fertilizer-treated plot, initial decreases in individual hydrocarbon concentration (Figures 6.67 through 6.72) were not accompanied by decreases in oil residue weight (Figure 6.15). Just the opposite was true on the untreated control plot. However, the greater overall decrease in individual hydrocarbon concentrations observed on the briquette fertilizer-treated plot (Figure 6.69) may have been related to the large decrease in oil residue weight following the July 29 sampling (Figure 6.14); in other words, biodegradation may have proceeded to a point where it changed the physical consistency of the oil, causing the degraded oil residues to be more easily removed from the beach matrix. Correspondingly, changes in oil composition on the untreated control plot may not have been great enough to elicit changes in oil residue weight.
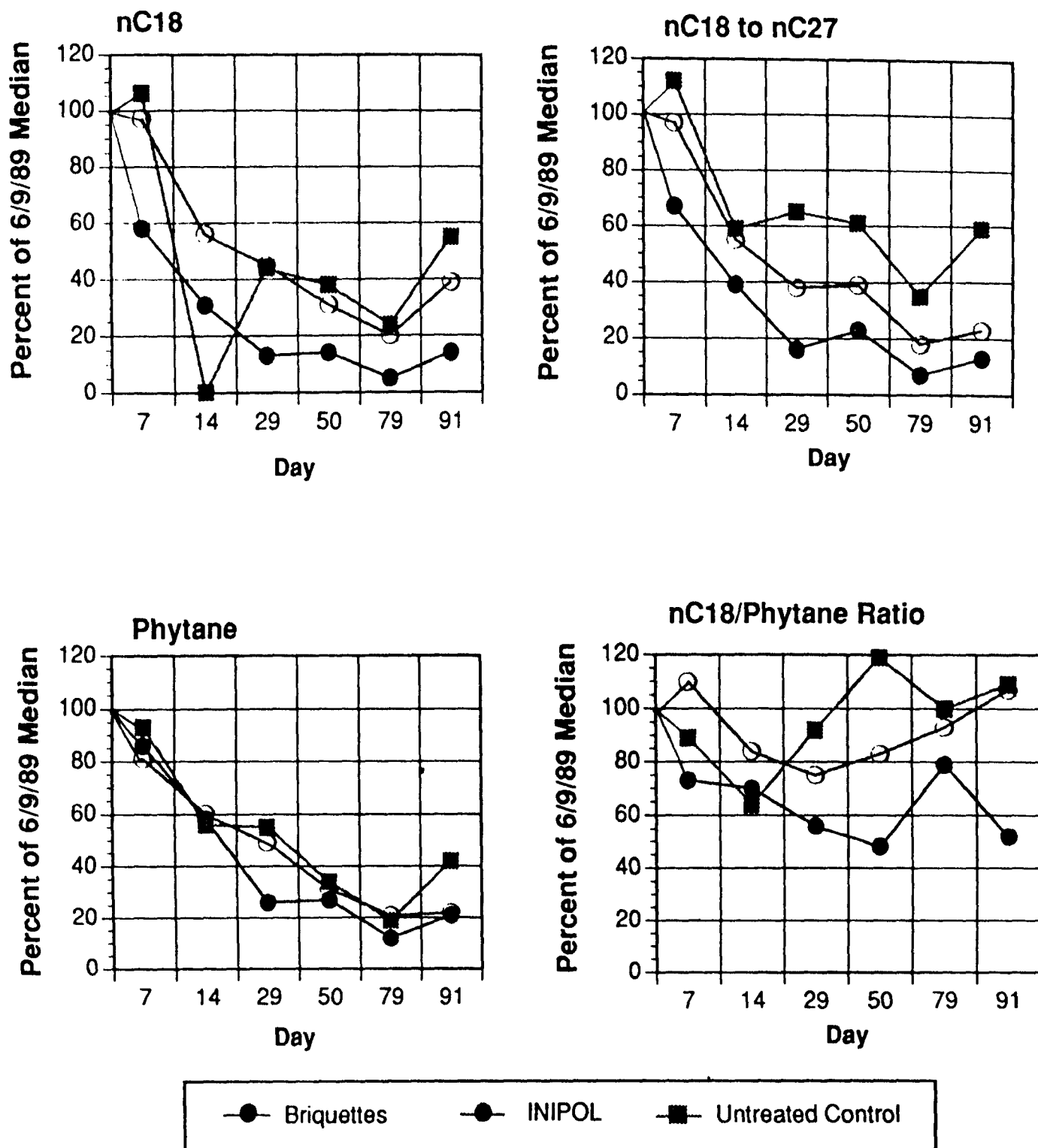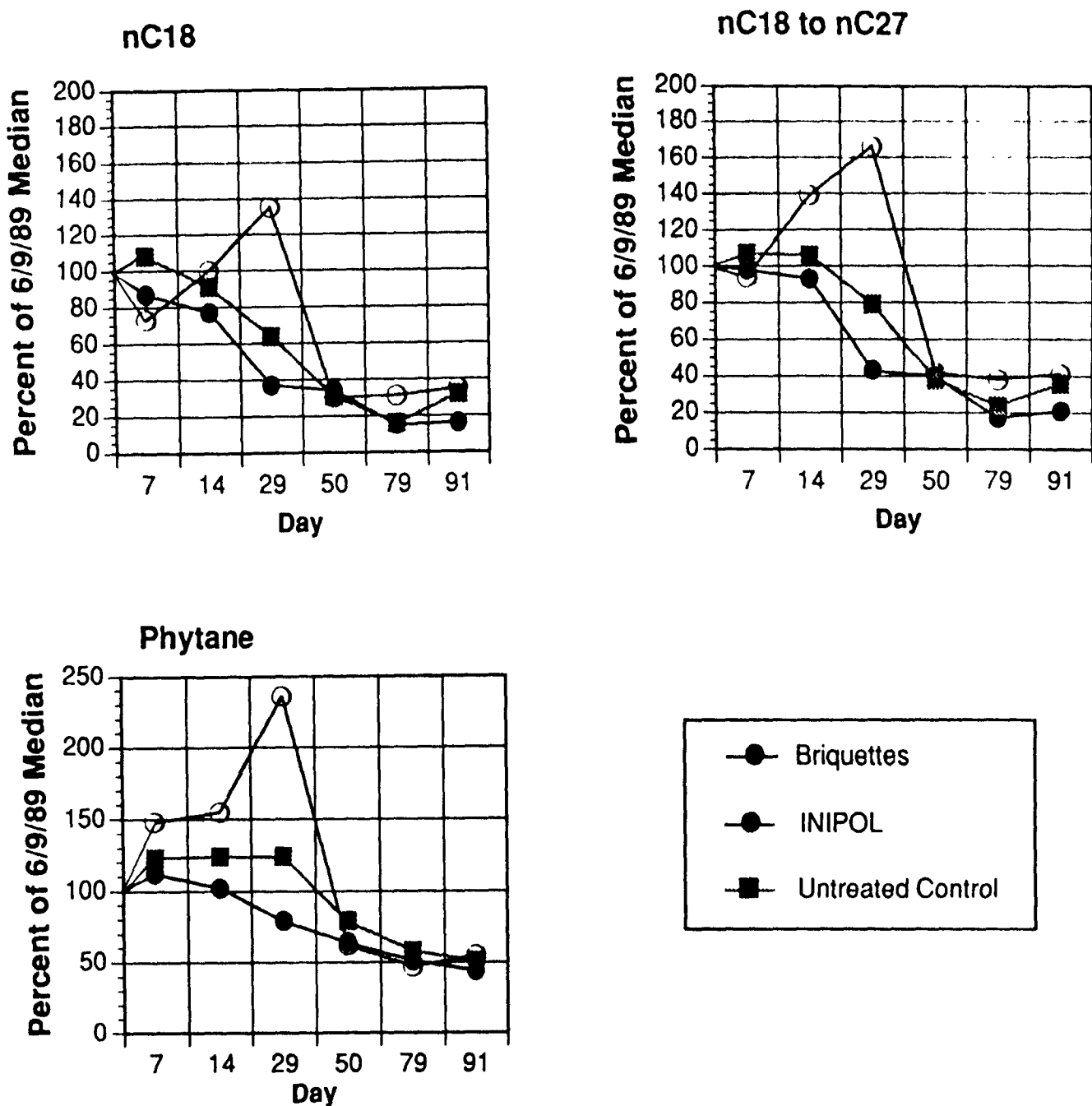
Figure 6.82. Change in the Median Residue Weight for Several Hydrocarbons Expressed as Percent of the 6/9/89 Median Over Time for the Briquette, INIPOL, and Untreated Control Beaches at Snug Harbor (Mixed Sand and Gravel Only). All Variability is not Shown Because the Actual Data Points are not Presented.

The situation on the INIPOL fertilizer-treated plot is more complicated to interpret. Again, initial increases in hydrocarbon concentrations were seen relative to the t=0 medians (Table 6.19). Possible explanations for this response were given above in the discussion of the cobble surface samples (page 161). Despite these unexplained increases in individual hydrocarbon concentrations, there was some decrease in the nC18/phytane ratio (Figure 6.81), suggesting that extensive biodegradation was occurring since it is unlikely that some nonbiological process would affect the nC18 and phytane hydrocarbons differentially. Rather dramatic changes occurred in hydrocarbon concentration following the July 8 (day 29) sampling date, allowing the INIPOL fertilizer-treated plot to, in essence, "catch up" with the other plots. However, these changes apparently were not large enough to affect any concomitant decrease in oil residue weights on the INIPOL fertilizer-treated plot (Tables 6.3 and 6.4).

In summary, for all types of beach material samples (cobble surface, mixed sand and gravel under cobble, and mixed sand and gravel only), it would appear that fertilizer application did enhance oil biodegradation. This was most obvious in the initial 29 days of the test on the plots treated with the fertilizer briquettes. Changes in oil composition, including the nC18/phytane ratios, were most extensive on these plots. However, this was not generally accompanied by significant changes in oil residue weight, and thus we would argue that changes in oil composition may not have been sufficient to cause large changes in oil residue for the fertilizer briquettes.

Significant changes did occur, however, in oil residue in the cobble surface samples with the INIPOL fertilizer application. Assuming the changes were due to biodegradation, by extrapolation one would expect large changes in oil composition. This was not the case; concentrations of individual hydrocarbons appeared to actually increase. It is possible that the INIPOL chemically caused a loss of oil residues, but laboratory experiments suggest this was not the case. In addition, no oil residues were detected in mussels suspended in cages just offshore the treatment area; with the amount of oil released it was expected that some should have bioconcentrated in the mussel tissue. It is more likely that components in the INIPOL interfered with the gas chromatographic analysis of the oil, possibly masking any changes in oil composition. If this was the case, it is also possible that the residual INIPOL materials contributed to the weight of the oil residues, again giving a conservative estimate of oil biodegradation.

TABLE 6.19. CHANGE IN HYDROCARBON COMPOSITION THROUGH TIME AT SNUG HARBOR, EXPRESSED IN PERCENT OF THE MEDIAN CONCENTRATION OF INDIVIDUAL HYDROCARBONS ON THE 6/9 SAMPLING[a]

Alkane	Beach Code	6/17	6/25	7/8	7/29	8/26	9/9
nC18	B	87	77	37	34	15	16
	I	73	100	135	30	31	35
	C	108	91	64	31	16	32
nC19	B	95	80	42	36	14	20
	I	85	112	145	33	31	39
	C	113	95	75	34	21	35
nC20	B	93	79	47	33	19	22
	I	89	123	182	38	31	41
	C	94	99	81	34	19	30
nC21	B	96	86	45	37	15	21
	I	89	128	163	40	35	40
	C	104	100	78	34	22	32
nC22	B	98	89	46	40	18	22
	I	93	143	187	39	38	48
	C	115	109	90	41	21	36
nC23	B	91	82	43	38	17	23
	I	97	149	166	49	46	40
	C	116	113	85	42	27	41
nC24	B	100	98	46	45	22	21
	I	110	143	174	44	44	49
	C	113	110	79	41	26	40
nC25	B	99	94	44	44	20	24
	I	114	153	172	39	42	39
	C	116	115	87	45	36	38
nC26	B	100	89	41	45	18	25
	I	133	162	157	47	41	40
	C	115	110	83	46	31	40
nC27	B	101	92	40	45	17	18
	I	117	176	177	55	27	13
	C	113	119	89	49	25	29
nC18 to nC27	B	98	93	43	40	17	21
	I	94	139	166	42	38	41
	C	107	106	79	38	24	36
Phytane	B	112	102	79	64	51	44
	I	148	155	236	62	47	55
	C	123	124	124	79	58	51

[a] B = Briquette fertilizer-treated plot; I = INIPOL fertilizer-treated plot; C = Untreated Control

Degradation Extent/Oil Residue Weight Relationships

During beach sampling it was obvious that globs of viscous, sticky oil were present in some areas. Where these globs were encountered, there was concern that spike concentrations of undegraded oil would mask evidence of degradation. Examination of the data indicated that changes in the nC17/pristane and nC18/phytane ratios were most apparent in the samples containing less total oil. This is reasonable if one realizes that at low concentrations, the surface area-to-oil residue weight ratio is large, as it is when oil is dispersed into the beach material as small droplets or films. Effectiveness of biodegradation will increase as the oil surface area increases. With higher concentrations of oil, the same degradation rate is probably occurring, but the surface area-to-oil amount is much less. Because the oil is in bigger globs, the degraded oil on the surface is diluted by the undegraded oil during sampling and homogenization. If this observation is valid, it should be possible to normalize the extent of degradation to the amount of oil present. Figures 6.83 through 6.86 show that when the nC17/pristane and nC18/phytane ratios are plotted against their respective residue weights, a direct relationship exists. This data is from plots prior to fertilizer treatment. Regression analysis of the data gave r-values around 0.8 (alpha = 0.0001). By comparing slopes of this relationship from two different sampling periods, the effect of biodegradation can be seen. The slopes increased by two- and three-fold over 2 weeks. With more degradation the slope will continue to steepen to a limit where the data points begin to cluster closer to the origin. This relationship may have application in further analyzing data from treated and untreated plots. Initial attempts to normalize the ratios with the oil residue weight to reduce variability of the data have, to date, been ineffective. The approach, however, seems promising and further work will evaluate its usefulness.

MICROBIOLOGY

Numbers of Oil-Degrading Bacteria

The relative numbers of oil-degrading bacteria present on beach materials were determined from parallel samples of beach material taken for oil chemistry. Sets of samples from the oleophilic fertilizer treated beach, the water-soluble fertilizer treated beach, and the untreated control beach were taken prior to fertilizer treatment, and on several dates after treatment. Numbers of oil-degrading bacteria were assessed by: serially diluting each sample in a minimal salts medium containing ammonium and phosphate ion; adding a small quantity of oil to each dilution; incubating the dilution tubes for 21 days; and then scoring the tubes for the absence or presence of biological growth and changes in the physical character of the oil.
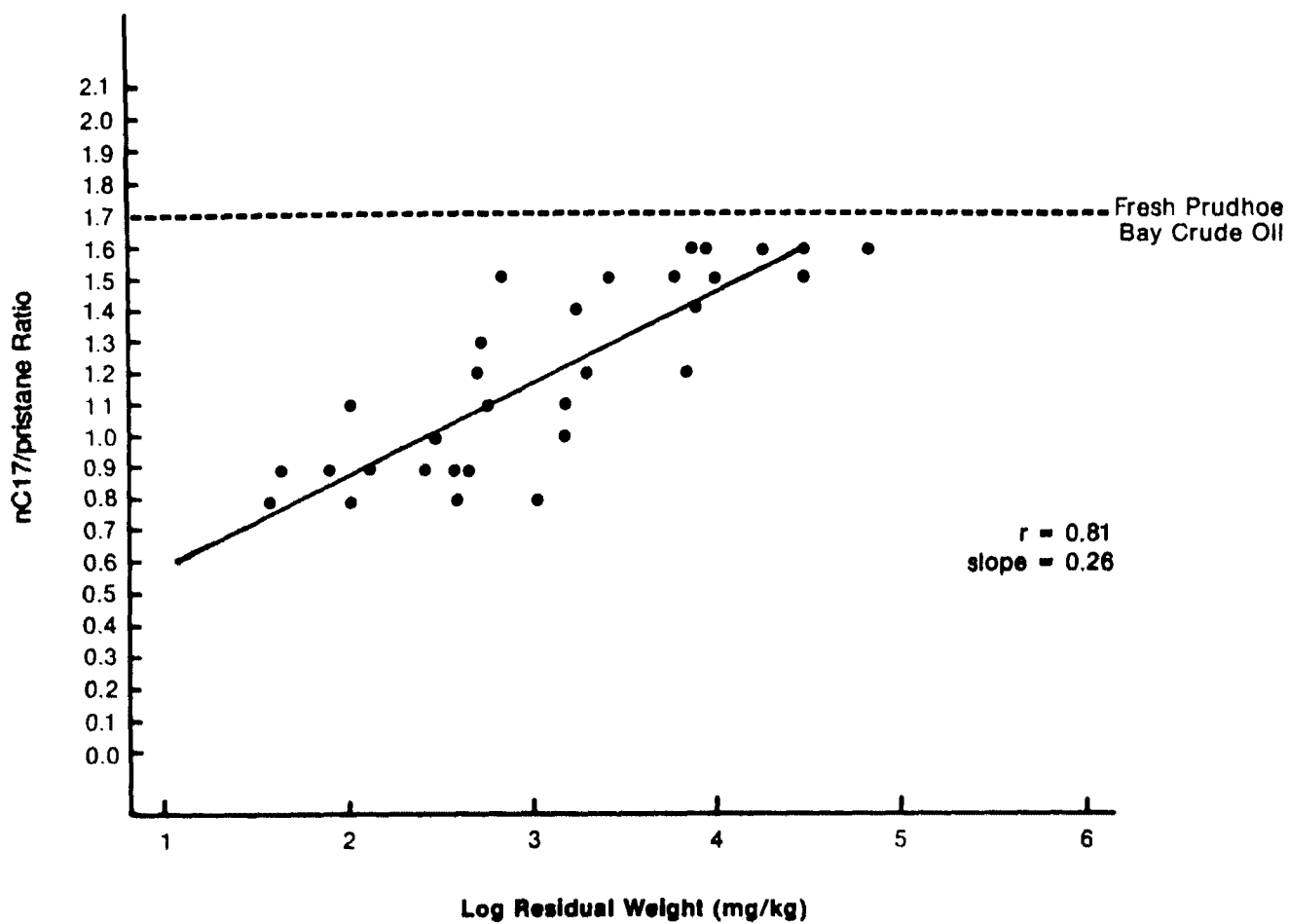
Figure 6.83. nC17/pristane Ratio versus Log$_{10}$ Residue Weight Two Weeks Before Fertilizer Application (5/28/89).

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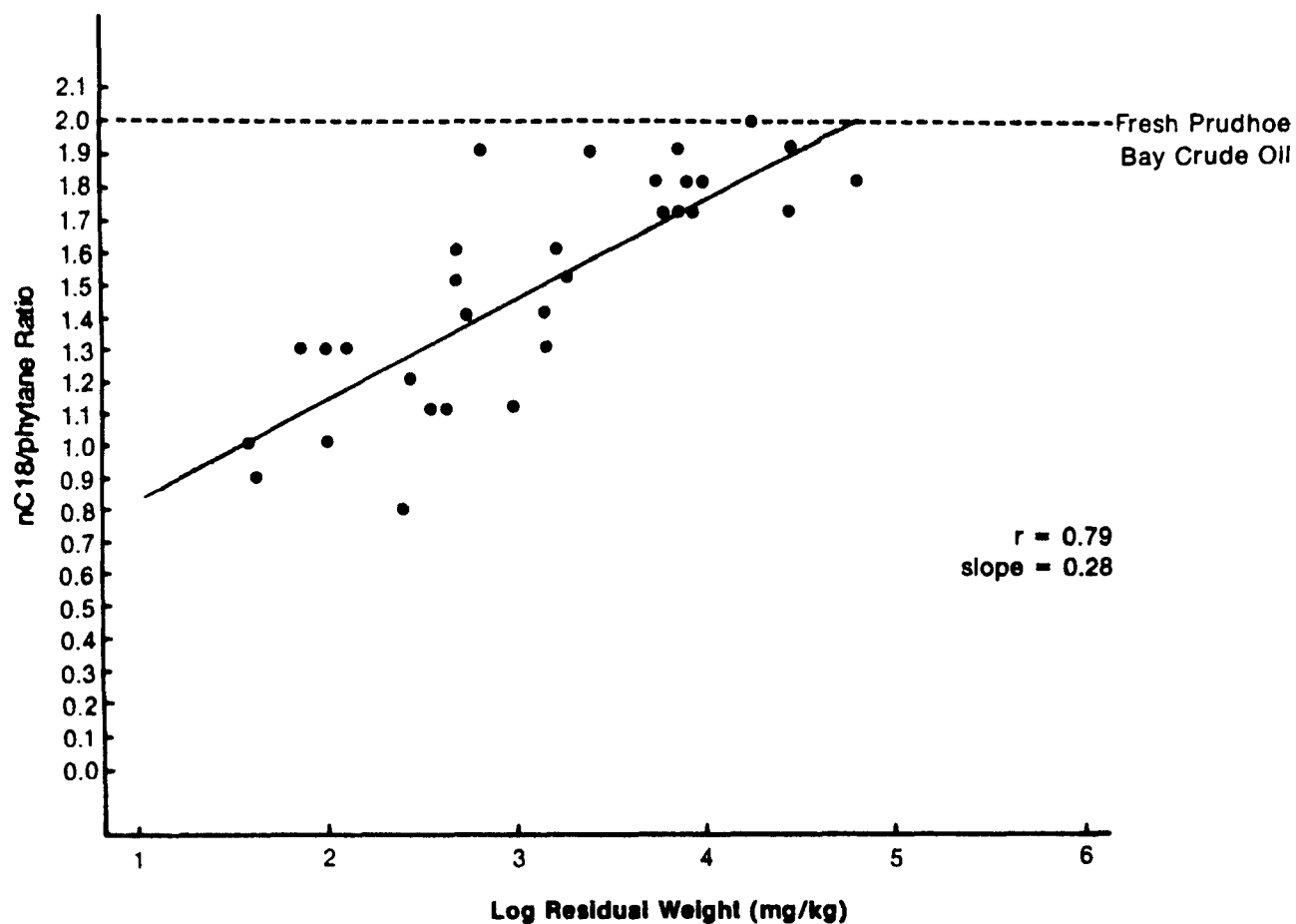
Figure 6.84. nC18/phytane Ratio versus Log$_{10}$ Residue Weight Two Weeks Before Fertilizer Application (5/28/89).
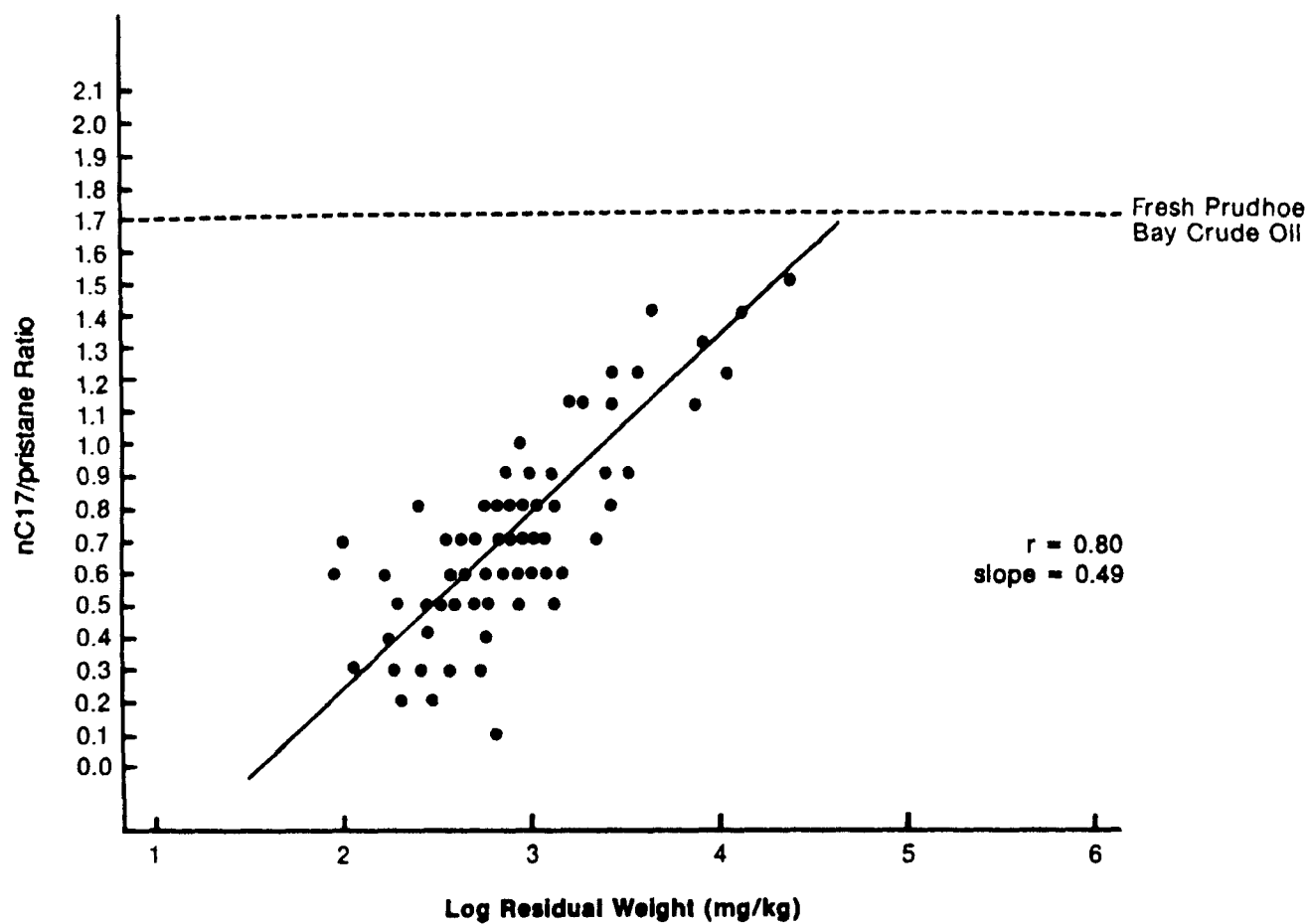
Figure 6.85. nC17/pristane Ratio Versus Log$_{10}$ Residue Weight at Time Zero of Fertilizer Application (6/8/89).
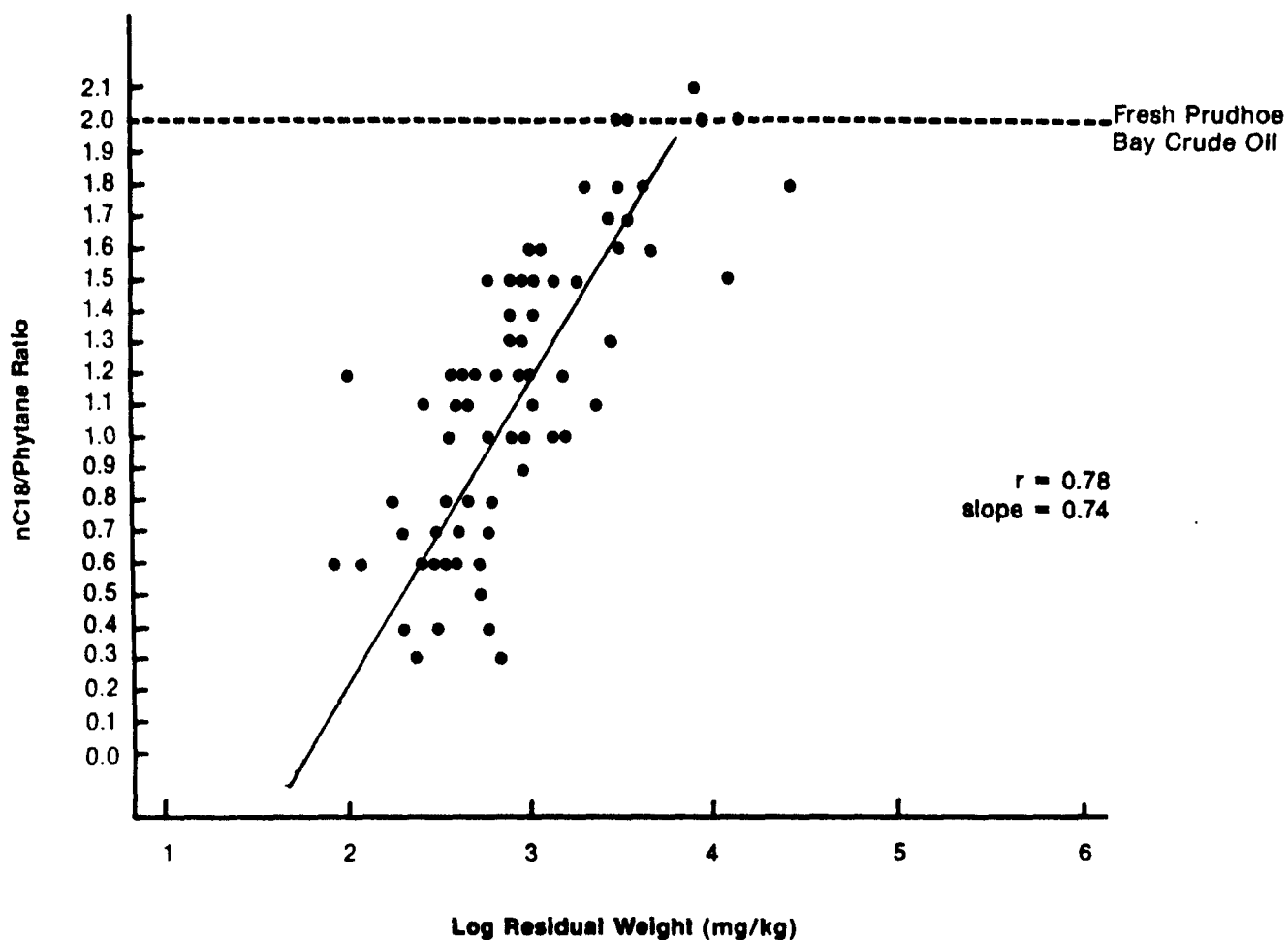
Figure 6.86.   nC18/phytane Ratio Versus Log_{10} Residue Weight at Time Zero of Fertilizer Application (6/8/89).

After the dilution tubes were scored, the relative bacterial populations were determined as follows: for a given dilution series, the highest positive dilution was assigned the last positive tube below which there was one or no negative tubes. Thus, a negative tube was ignored only when the first negative tube was followed by a positive tube. It is believed that this procedure did not significantly bias the results, especially when focusing on relative comparisons between treated and untreated control plots. In the first three sampling periods the dilutions were carried to a maximum factor of 10^8, and in the last sampling period to a maximum factor of 10^{10}.

The results shown in Table 6.20 are expressed as the $\log_{10}$ of the dilution factors used. In evaluating these results, it is important to recognize that a significant number of the dilution series were not high enough (i.e., the highest dilution series did not contain all negatives). Whenever samples have been under-diluted, the normal summary statistics (such as the mean or standard deviation) are biased. Also, whenever the median is the maximum dilution value, no good estimate of the average is available. This situation occurred twice, so in these cases the true average is larger than the median. However, when there are only a few maximum dilution values, the mean and standard deviation will not be overly biased. In these cases the bias in the mean may be less than the possible error from using the median. The possible change (error) in the median is 0.5 for the gain or loss of one data point or for an error in reading any dilution series by one tube.

There was an upward trend in number of organisms from 10^5 to 10^7 per unit volume over the sampling period for the control beach. A similar pattern was observed for the beach treated with water-soluble fertilizer, except the number increased to 10^{10} for the last sampling date. The reasons for this increase are not known. In contrast, a transient increase in bacteria numbers was observed with samples from the beach treated with oleophilic fertilizer. This increase might be associated with the transient availability of nutrients released from the oleophilic fertilizer.

ECOLOGICAL MONITORING

The monitoring component of the project was designed to identify ecological effects of nutrients added to the shore zone on planktonic microorganisms. Sampling stations were established in nearshore locations next to both treated and untreated control beaches in Snug Harbor (see Sections 3 and 4) and at locations outside Snug Harbor. Samples were collected on 9 occasions; once prior to the addition of fertilizer, 2 days after addition, and 1, 2, 3, 4, 5, 6, and 8 weeks after addition. After week 5, the stations 10 m from shore were no longer sampled in order to accommodate the workload

TABLE 6.20. RELATIVE LEVELS OF OIL-DEGRADING MICROORGANISMS
IN SNUG HARBOR MIXED SAND AND GRAVEL TEST PLOTS[a,b]

UNTREATED CONTROL BEACH

	Pre-Treatment	6/17/89	6/24/89	7/8-9/89
N	22	21	20	19
n>max	0	8	1	4
Median	5	6	6	7
Mean	5.18	6.33	6.05	7.58
SD	0.91	1.53	0.83	1.61
SD-Mean	0.19	0.33	0.19	0.37

WATER-SOLUBLE FERTILIZER-TREATED BEACH

	Pre-Treatment	6/17/89	6/24/89	7/8-9/89
N	20	21	18	21
n>max	2	3	0	16
Median	5	6	6	10+
Mean	5.65	6.29	5.78	9.48
SD	1.04	1.10	0.65	1.12
SD-Mean	0.23	0.24	0.15	0.24

OLEOPHILIC FERTILIZER-TREATED BEACH

	Pre-Treatment	6/17/89	6/24/89	7/8-9/89
N	20	20	20	21
n>max	2	11	2	0
Median	6	8+	6	6
Mean	5.75	7.00	6.05	5.95
SD	1.29	1.21	1.10	0.67
SD-Mean	0.29	0.27	0.25	0.15

[a] The tabulated results show the number of samples run per beach (N), the number of serial dilutions still showing positive results at the highest dilution (n>max), the median, the mean, the standard deviation (SD), and the standard deviation of the mean (SD-Mean).

[b] All results are expressed as the log_{10} of the dilution factors used.

from an additional study site. Data analyzed after week 5 indicated no significant loss in assessment capability resulted from this decision.

Nutrients from Nearshore Waters

None of the nutrient concentrations increased in waters adjacent to treated shoreline compared to the control shoreline, as illustrated by the ammonia and phosphorus data in Tables 6.21 through 6.24. These data provide evidence that fertilizers applied to the Snug Harbor shoreline either remained within the matrix as applied, were taken up by microbial biomass, or were diluted to background concentrations once they reached the shoreline. In any case, the potential for stimulating plankton biomass from nutrient enrichment along the shoreline was not evident from these data.

Chlorophyll Analyses

Chlorophyll analyses of phytoplankton samples were used to monitor changes in the abundance of algae. Nutrient enrichment could stimulate algal growth in Snug Harbor, and increased chlorophyll concentrations would be evidence that nutrients had washed from the beach and had been incorporated into algal biomass. None of the chlorophyll data indicated that algal populations within Snug Harbor were stimulated by fertilizer applications (Figure 6.87). Results demonstrate that nearshore concentrations were similar to those offshore; differences between samples collected near treated beaches and reference areas were not ecologically significant. Differences observed between nearshore (1 m) and offshore (10 m) samples and fertilized and reference shoreline samples were within the expected range of day-to-day variation (Tables 6.25 and 6.26). All chlorophyll values were within the expected range for Prince William Sound plankton communities.

Phytoplankton Primary Productivity

Photosynthetic production by phytoplankton is estimated by the incorporation of ^{14}C-bicarbonate, providing a functional measure of the photosynthetic activity of algal cells. It allows an evaluation of whether the algal population sampled is viable and active, nutrient limited, or enriched. Comparisons of photosynthetic rates obtained on different sampling dates are not valid because the light conditions during incubation could have been different enough to significantly affect the daily productivity estimate. Only treated-versus-control comparisons are valid for each sampling date. On several dates, primary productivity estimates from stations near fertilized shorelines were significantly

TABLE 6.21. AMMONIA NITROGEN (μ N/L) FROM NEARSHORE WATER OVER GRAVEL BEACHES AT SNUG HARBOR. MEAN OF FOUR REPLICATES (STANDARD DEVIATION). (METHOD DETECTION LIMIT = 0.13 μ N/L.)

Sample Date	Untreated Control (Rodney Beach)		Oleophilic (Otter Beach)		Water-Soluble (Otter Beach)	
	1 m	10 m	1 m	10 m	1 m	10 m
6/10/89	1.5 (0.05)	1.5 (0.08)	1.6 (0.05)	1.7 (0.06)	1.5 (0.22)	1.8 (0.17)
6/14/89	0.68 (0.10)	0.65 (0.05)	0.52 (0.09)	0.58 (0.10)	0.61 (0.08)	0.58 (0.10)
6/21/89	0.92 (0.03)	1.02 (0.06)	0.74 (0.03)	0.83 (0.05)	0.73 (0.03)	0.74 (0.06)
6/28/89	0.21 (0.06)	0.15 (0.02)	0.13 (0.00)	0.20 (0.10)	0.13 (0.00)	0.20 (0.14)
7/5/89	0.51 (0.11)	0.52 (0.03)	0.56 (0.09)	0.57 (0.10)	0.74 (0.16)	0.53 (0.09)
7/12/89	0.80 (0.32)	0.73 (0.19)	0.57 (0.11)	0.50 (0.05)	0.63 (0.08)	0.96 (0.57)
7/23/89	0.13 (0.00)	--[a] --	0.13 (0.00)	-- --	0.13 (0.00)	-- --
8/9/89	0.13 (0.00)	-- --	0.13 (0.00)	-- --	0.13 (0.00)	-- --

[a] -- = Sample not collected.

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TABLE 6.22. AMMONIA NITROGEN (μ N/L) FROM NEARSHORE WATER OVER COBBLE BEACHES AT SNUG HARBOR. MEAN OF FOUR REPLICATES (STANDARD DEVIATION). (METHOD DETECTION LIMIT = 0.13 μ N/L.)

Sample Date	Untreated Control (Fred Beach)		Oleophilic (Seal Beach)		Water-Soluble (Seal Beach)	
	1 m	10 m	1 m	10 m	1 m	10 m
6/10/89	2.1 (0.12)	1.8 (0.00)	1.5 (0.05)	0.5 (0.10)	1.4 (0.27)	1.4 (0.08)
6/14/89	0.73 (0.03)	0.70 (0.08)	0.45 (0.06)	0.55 (0.12)	0.64 (0.06)	0.48 (0.06)
6/21/89	0.99 (0.08)	0.91 (0.06)	0.96 (0.04)	0.82 (0.03)	0.87 (0.09)	0.88 (0.10)
6/28/89	0.24 (0.06)	0.35 (0.26)	0.22 (0.13)	0.13 (0.00)	0.22 (0.11)	0.18 (0.07)
7/5/89	0.61 (0.12)	0.65 (0.19)	0.52 (0.03)	0.50 (0.05)	0.44 (0.21)	0.47 (0.05)
7/12/89	0.62 (0.18)	0.70 (0.20)	0.79 (0.16)	0.75 (0.14)	0.86 (0.17)	0.78 (0.08)
7/23/89	0.13 (0.00)	--[a]	0.13 (0.00)	--	0.13	--
8/9/89	0.13 (0.00)	--	0.13 (0.00)	--	0.13	--

[a] -- Sample not collected.

TABLE 6.23. PHOSPHATE (μ P/L) FROM NEARSHORE WATER OVER GRAVEL BEACHES AT SNUG HARBOR. MEAN OF FOUR REPLICATES (STANDARD DEVIATION). (METHOD DETECTION LIMIT = 0.20 μ P/L FOR SAMPLE DATE 6/10/89, 0.02 μ P/L THEREAFTER.)

Sample Date	Untreated Control (Rodney Beach)		Oleophilic (Otter Beach)		Water-Soluble (Otter Beach)	
	1 m	10 m	1 m	10 m	1 m	10 m
6/10/89	0.20 (0.00)	0.20 (0.00)	0.34 (0.27)	0.20 (0.00)	0.20 (0.00)	0.26 (0.12)
6/14/89	0.10 (0.00)	0.13 (0.03)	0.18 (0.04)	0.15 (0.00)	0.15 (0.04)	0.12 (0.05)
6/21/89	0.44 (0.00)	0.40 (0.03)	0.29 (0.04)	0.28 (0.11)	0.34 (0.03)	0.35 (0.04)
6/28/89	0.25 (0.00)	0.25 (0.00)	0.15 (0.03)	0.17 (0.02)	0.20 (0.00)	0.16 (0.00)
7/5/89	0.27 (0.04)	0.27 (0.04)	0.36 (0.04)	0.23 (0.03)	0.37 (0.05)	0.28 (0.04)
7/12/89	0.23 (0.03)	0.29 (0.03)	0.22 (0.04)	0.32 (0.03)	0.25 (0.03)	0.22 (0.00)
7/23/89	0.08 (0.00)	--[a] --	0.08 (0.00)	-- --	0.10 (0.03)	-- --

[a] -- = Sample not collected.

TABLE 6.24. PHOSPHATE (μ P/L) FROM NEARSHORE WATER OVER COBBLE BEACHES AT SNUG HARBOR. MEAN OF FOUR REPLICATES (STANDARD DEVIATION). METHOD DETECTION LIMIT = 0.20 μ P/L FOR SAMPLE DATE 6/10/89, 0.02 μ P/L THEREAFTER.)

Sample Date	Untreated Control (Fred Beach)		Oleophilic (Seal Beach)		Water-Soluble (Seal Beach)	
	1 m	10 m	1 m	10 m	1 m	10 m
6/10/89	0.22 (0.03)	0.20 (0.00)	0.20 (0.00)	0.20 (0.00)	0.22 (0.03)	0.20 (0.00)
6/14/89	0.16 (0.02)	0.15 (0.00)	0.15 (0.00)	0.12 (0.03)	0.14 (0.04)	0.14 (0.06)
6/21/89	0.36 (0.02)	0.31 (0.03)	0.35 (0.04)	0.25 (0.03)	0.26 (0.00)	0.27 (0.04)
6/28/89	0.18 (0.02)	0.28 (0.03)	0.16 (0.04)	0.15 (0.03)	0.20 (0.04)	0.24 (0.05)
7/5/89	0.29 (0.05)	0.30 (0.04)	0.32 (0.03)	0.25 (0.03)	0.34 (0.03)	0.30 (0.07)
7/12/89	0.38 (0.00)	0.34 (0.03)	0.25 (0.03)	0.23 (0.05)	0.25 (0.06)	0.22 (0.00)
7/23/89	0.10 (0.03)	--[a] --	0.09 (0.01)	-- --	0.09 (0.01)	-- --

[a] -- = Samples not collected.

TABLE 6.25. TIDAL VARIATION IN MEASUREMENTS OF BACTERIAL ABUNDANCE
AND PLANKTON CHLOROPHYLL *a* FOR SAMPLING STATIONS AT SNUG HARBOR
ON 7/26-27/89. A SEQUENTIAL SERIES OF SAMPLES WAS COLLECTED
OVER A 24-HOUR PERIOD AT HIGH, MID, AND LOW TIDE.
REFER TO FIGURE 3.2 FOR SAMPLE STATION LOCATIONS

	Bacterial Enumeration (cells x 10^9/L)						
Station	High	Mid	Low	Mid	High	Mid	Low
Untreated Control	0.75	0.63	0.66	0.58	0.66	0.61	0.75
Gravel	(0.06)	(0.03)	(0.05)	(0.02)	(0.04)	(0.04)	(0.04)
Gravel	0.70	0.67	0.57	0.62	0.64	0.73	0.74
Water-sol.	(0.04)	(0.06)	(0.04)	(0.14)	(0.04)	(0.01)	(0.02)
Cobble	0.73	0.63	0.60	0.63	0.66	0.74	0.74
Water-sol.	(0.02)	(0.02)	(0.03)	(0.03)	(0.01)	(0.03)	(0.02)
	μg Chlorophyll *a*/L						
Untreated Control	0.29	1.17	0.82	0.62	0.87	0.70	1.00
Gravel	(0.49)	(0.30)	(0.09)	(0.08)	(0.06)	(0.08)	(0.09)
Gravel	1.02	0.89	0.80	0.77	0.90	0.88	0.65
Water-sol.	(0.26)	(0.340	(0.07)	(0.06)	(0.13)	(0.11)	(0.18)
Cobble	0.76	0.42	0.92	0.89	0.90	0.95	0.79
Water-sol.	(0.04)	(0.11)	(0.12)	(0.13)	(0.04)	(0.17)	(0.07)

TABLE 6.26. TIDAL VARIATION IN MEASUREMENTS OF BACTERIAL ABUNDANCE AND PLANKTON CHLOROPHYLL *a* FOR SAMPLING STATIONS AT PASSAGE COVE ON 8/7/89. VALUES ARE MEANS OF 4 REPLICATES WITH STANDARD DEVIATION (). A SEQUENTIAL SERIES OF SAMPLES AS COLLECTED OVER ONE TIDE AT HIGH, MID, AND LOW TIDE. REFER TO FIGURE 3.3 FOR SAMPLE STATION LOCATIONS

	Bacterial Enumeration (cells x 10^9/L)		
Station	High	Mid	Low
Station 3, 0.5 m	0.18 (0.01)	0.24 (0.04)	0.26 (0.04)
Station 3, 5.0 m	0.57 (0.03)	0.25 (0.020	0.52 (0.08)
Station 5, 0.5 m	0.27 (0.01)	0.50 (0.07)	0.24 (0.02)
	μg Chlorophyll *a*/L		
Station 3, 0.5 m	0.46 (0.12)	0.40 (0.03)	0.37 (0.19)
Station 3, 5.0 m	0.38 (0.55)	0.50 (0.03)	0.16 (0.05)
Station 5, 0.5 mn	0.60 (0.28)	0.46 (0.04)	0.15 (0.39)
Station 7, 0.5 m	0.64 (0.19)	0.46 (0.11)	0.65 (0.40)
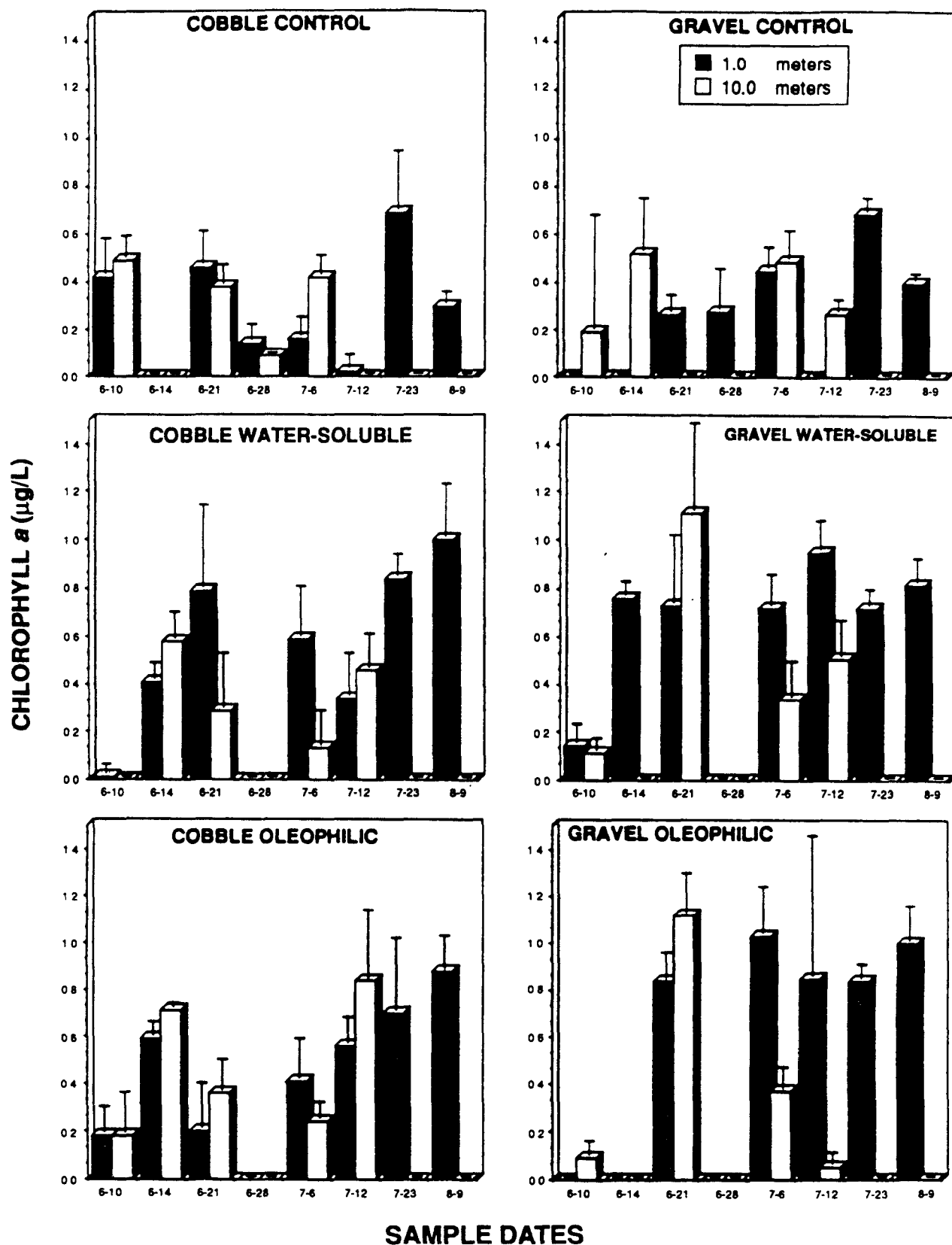
Figure 6.87. Phytoplankton Chlorophyll Data (mg Chlorophyll *a*/L) from Water Samples Collected Along Cobble and Gravel Shorelines at Snug Harbor Following June 7 and 8, 1989, Fertilizer Additions to Gravel Shorelines. Values are Means (+SD) of 4 Replicates; Dark Bars are for Sample Sites 1 m Offshore, Open Bars are For Sites 10 m Offshore. Refer to Figure 3.2 for Station Locations.

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greater than control values using statistical comparisons (Figure 6.88). However, these differences generally were less than a factor of 2, inconsistent through time, and within the range of expected ecological variability. If small changes in daily primary productivity were occurring, the lack of a change in chlorophyll content suggests that the increased biomass associated with increased productivity was not accruing faster than the dilution and transport of water masses associated with tidal exchange for the basin.

Bacterial Abundance

Abundance of bacteria in the water column samples from Snug Harbor is reported in Figure 6.89. Mean bacterial abundances varied from 0.21 to 2.49 X 10^9 cells per liter. One week after the nutrient additions, numbers were higher than Day 2 values for the fertilized shorelines, but the values for control shorelines did not change from Day 2 to Week 1. Because the values for treated shorelines did not increase beyond control shoreline values, the changes, by themselves, are not considered ecologically significant. Differences observed between treated and control areas on other dates were within the range of natural variability shown in Tables 6.25 and 6.26. Bacterial abundance showed no trends associated with shoreline treatments, nearshore versus offshore comparisons, or changes through time over the monitoring period.

Bacterial Production

Since the presence of cells alone may not represent the viability of planktonic microbes, bacterial production estimates allow an evaluation of functional activity of the bacterial community and the effect of nutrient enrichments. As seen in the bacterial abundance data, there were no consistent changes or trends in bacterial production measurements that can be associated with fertilizer application to the shoreline (Figure 6.90). An inspection of the data shows greater productivity during the first two sampling periods compared to subsequent sampling. This was seen at control samples as well as treated sites, indicative of a seasonal trend rather than a treatment effect. None of these differences appeared to be ecologically significant.

PRIMARY PRODUCTIVITY (mg C/m³/hr)

6-10-89

6-14-89

6-21-89

6-28-89
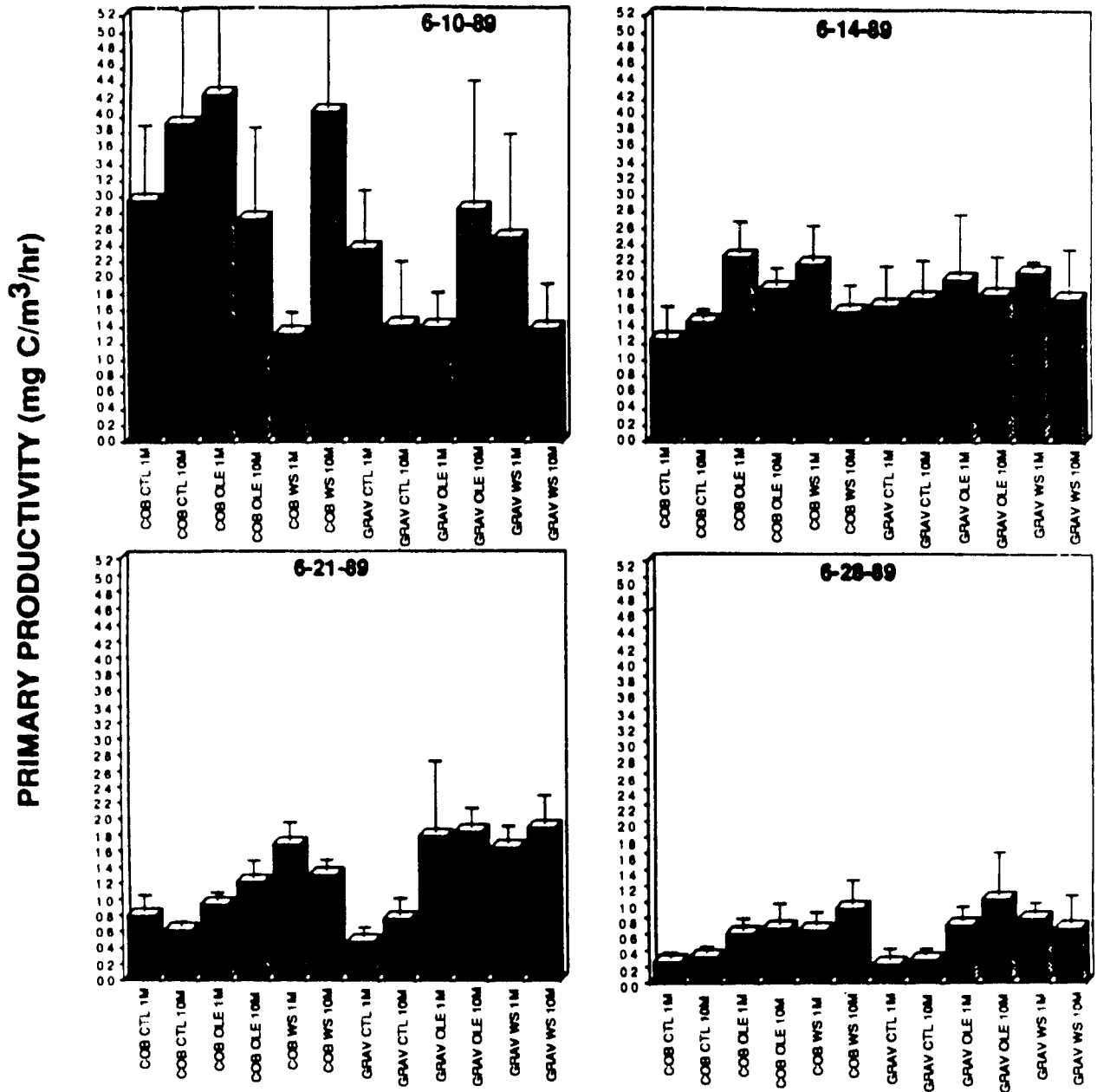
Figure 6.88. Primary Productivity Estimates (From ¹⁴C Uptake; mg C/m³/hour) For Phytoplankton Samples From Snug Harbor at Various Sample Dates Following the June 7 and 8, 1989, Fertilizer Additions Along Cobble and Gravel Shorelines. Values are Means (+SD) of 4 Replicates. Refer to Figure 3.2 for Station Locations.
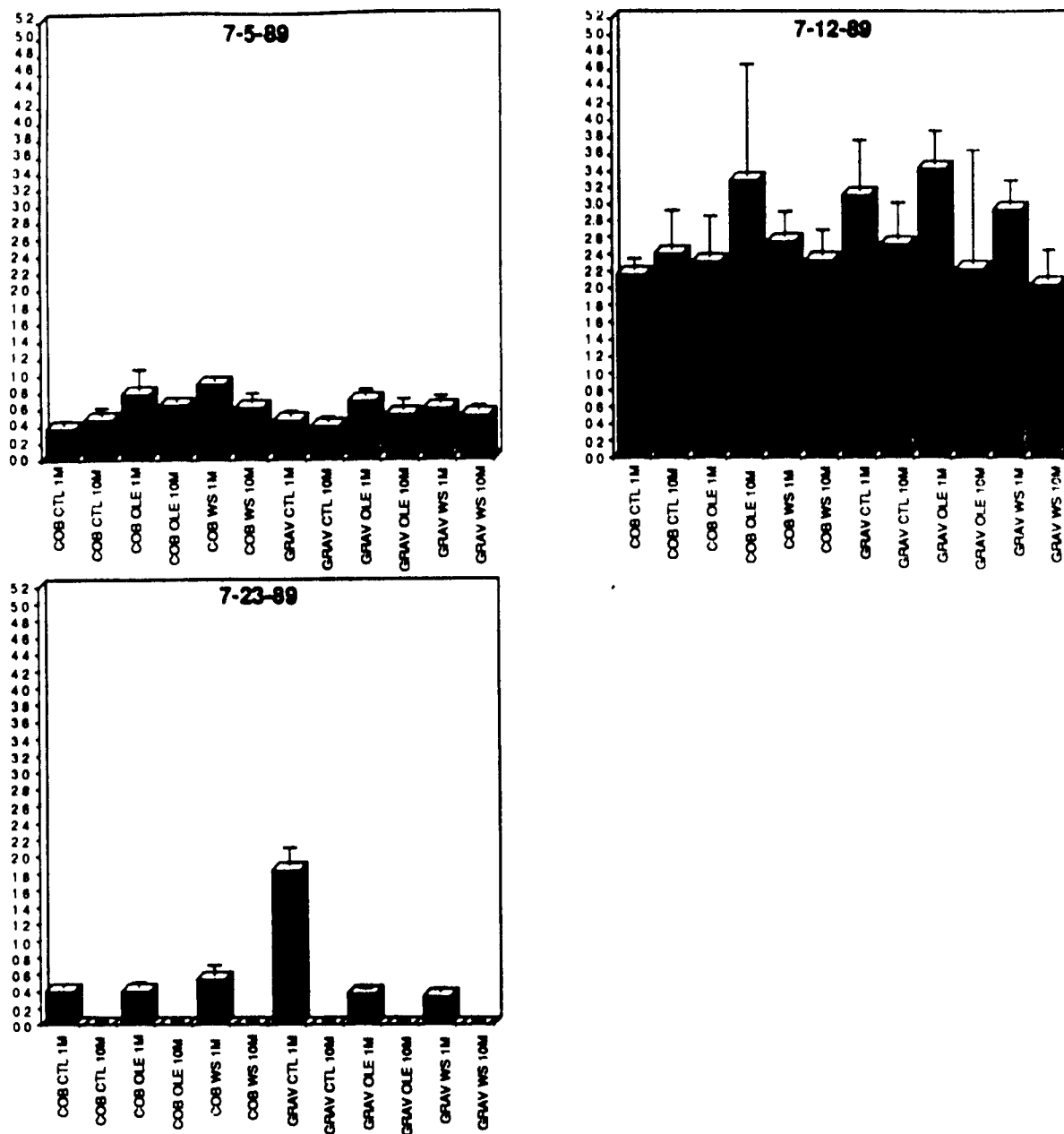
Figure 6.88.   (Continued)
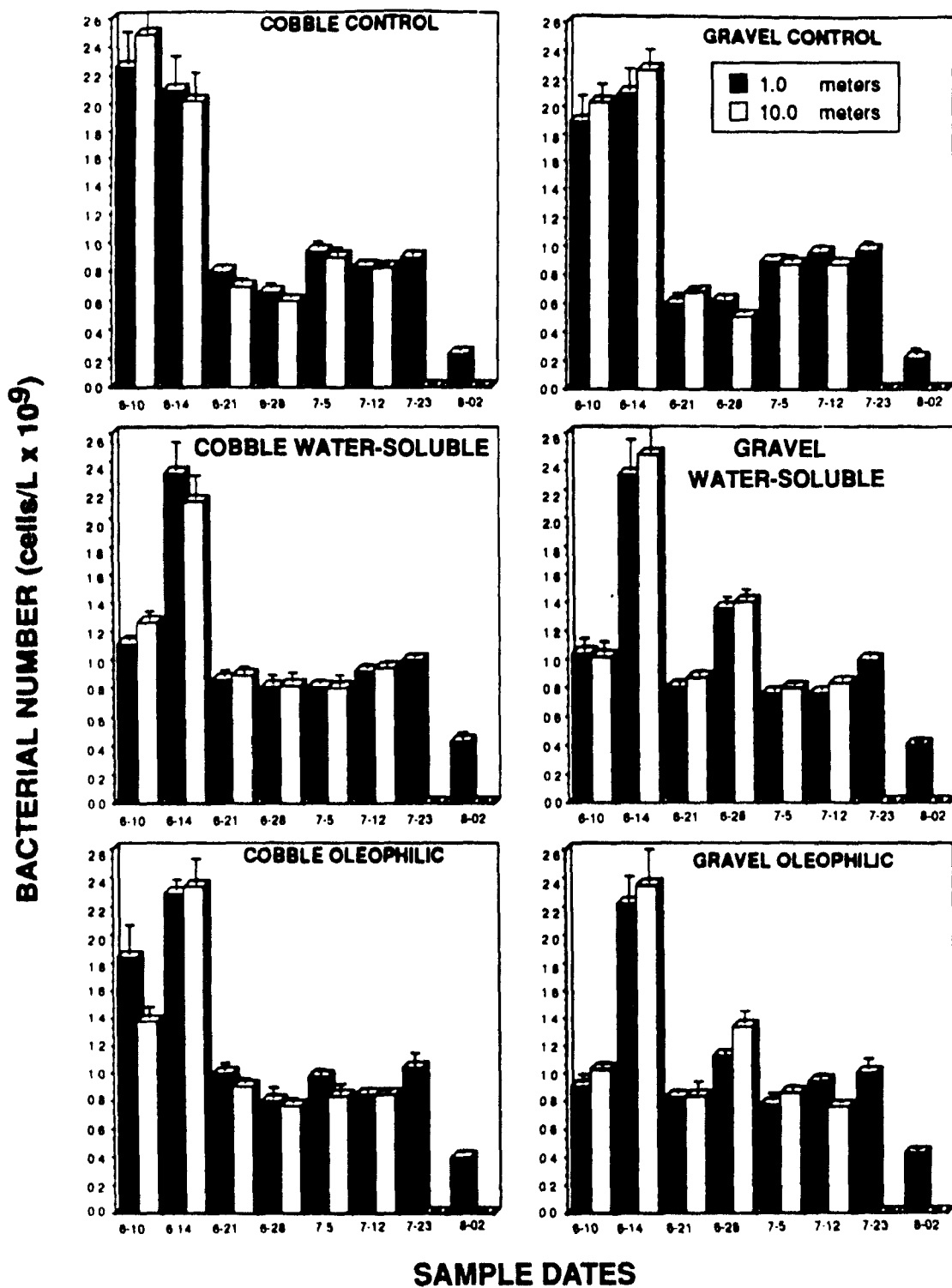
Figure 6.89. Abundance of Bacteria (cells x 10^9/L) From Water Samples Taken Along Cobble and Gravel Shorelines on Various Sample Dates Following the June 7 and 8, 1989, Fertilizer Additions to Snug Harbor Shorelines. Plotted Values are Means (+ SD) of 4 Replicates; Dark Bars are For Sample Sites 1 m Offshore, Open Bars are For Sites 10 m Offshore. Refer to Figure 3.2 for Station Locations.
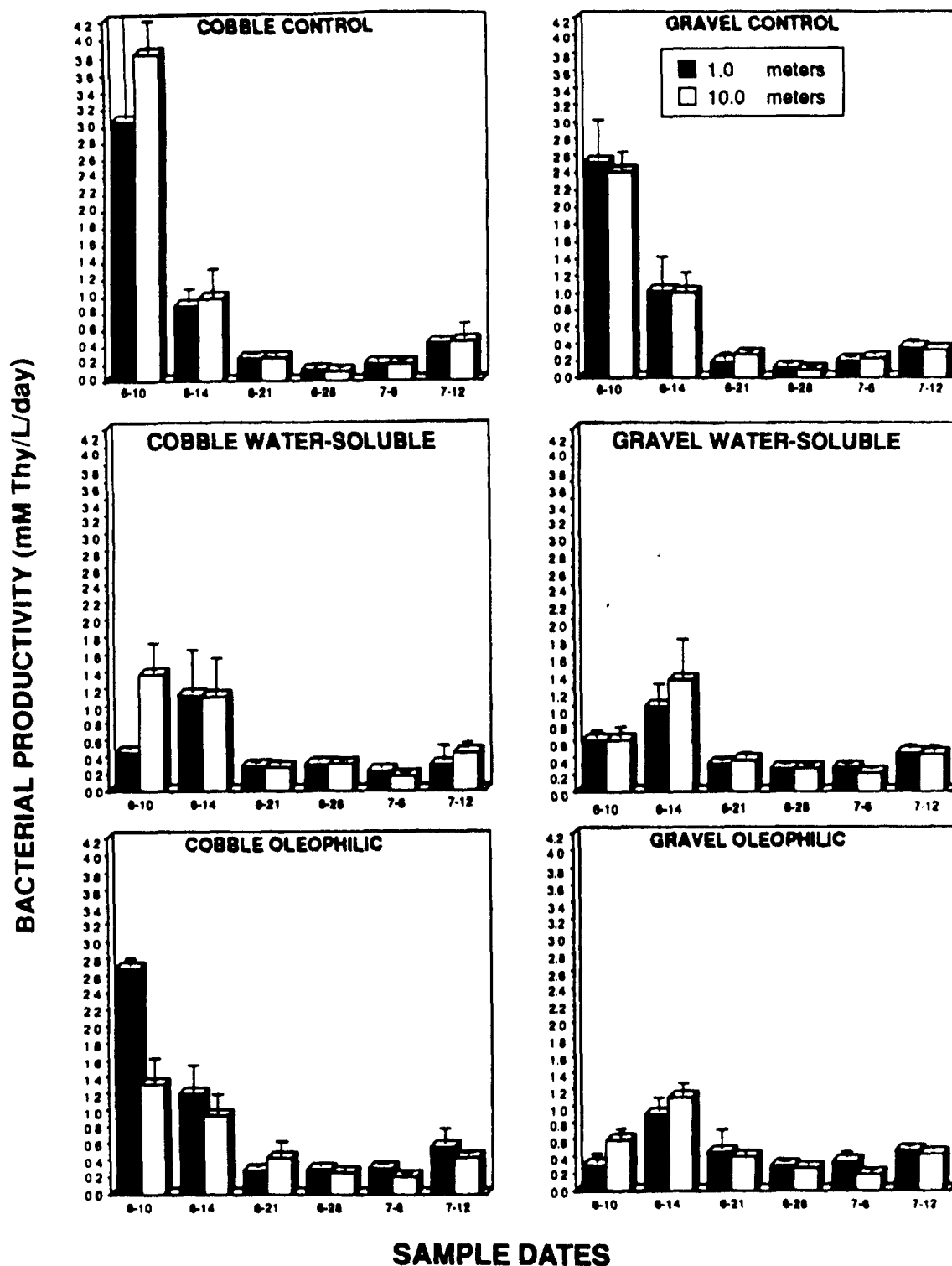
Figure 6.90. Bacterial Productivity, as Measured by Tritiated Thymidine Uptake (mM Thymidine/L/day), for Bacterial Samples Collected on Various Sample Dates Adjacent to Cobble and Gravel Shorelines at Snug Harbor. Fertilizer Additions Were Completed on June 7 and 8, 1989. Plotted Values are Means (+SD) of 4 Replicates; Dark Bars Are For Sample Sites 1 m Offshore, Open Bars Are For Sites 10 m Offshore. Refer to Figure 3.2 for Station Locations.

Caged Mussels

None of the mussel tissue samples had detectable residues of PAHs (Table 6.27). Application of water-soluble nutrients (water) or oleophilic nutrients (Oleo) did not increase the input of petroleum products into the nearshore zone to the extent that residues became detectable in nearshore mussels. Oil components were also not detected either from the control areas. The detection limit for these samples was low enough to identify the presence of PAHs before they might become an ecotoxicological problem. Bioaccumulation of PAHs by nearshore mussels was not detected at Snug Harbor either due to a lack of PAH input, or mixing and dilution of PAHs that did make it into the nearshore zone.

TABLE 6.27. TOTAL PAH'S (μG/G) IN CAGED MUSSELS AT SNUG HARBOR
AT 6 STATIONS OVER TIME

Date	Cobble Seal		Sand Otter		Cobble Fred Nails Control	Sand Rodney Control	Detection Limit (μg/g)	No. of Replicates Analyzed
	Water	Oleo	Water	Oleo				
6/21	ND	ND	ND	ND	ND	ND	0.20	4
6/28	ND	ND	ND	ND	ND	ND	0.20	4
7/5	ND	ND	ND	ND	ND	ND	0.20	4
7/10	ND	ND	ND	ND	ND	ND	0.20	4
7/23	ND	ND	ND	ND	ND	ND	0.20	4
8/10	ND	ND	ND	ND	ND	No sample	0.20	4
8/27	ND	ND	ND	ND	No sample	ND	0.05	4

ND = None detected

Problems were encountered using a small sample size (3 mussels/sample collected) and should have been larger (10 mussels/sample collected). In addition, the number of "time zero" mussels collected should have been equal to the number of mussels set out in all the test sites at the beginning of the test. This would ensure enough tissue for analytical method development and validation at the beginning of the test, and enough for quality assurance analysis for the duration of the test.

MICROCOSM STUDIES

Microcosm tank studies were performed in the summer of 1989 to simulate the field demonstration project as a protection against possible loss of data, such as through a major storm event or some other unforseen complications, and to provide a potential basis from which scale-up decisions could be made. These studies were therefore designed to test the effects of the fertilizer treatments under controlled conditions.

Tank Microcosms

Mixed sand and gravel microcosms were sampled 22 days post application (July 7) and cobble microcosms were sampled 26 days post application and 41 days post application (July 11 and 26). Visual observations at the time of sampling indicated that the oleophilic fertilizer-treated cobble microcosms appeared to have the least surface oil on the cobble surfaces, but the difference with other treated and control microcosms was not dramatic. Where oil was present it appeared mottled, suggesting that the oil on the surface had been partially removed or degraded. Oil was apparent under the rocks, but it was very black and viscid. This consistency appeared to be due to the oleophilic fertilizer dissolving into the oil.

The amount of surface oil in the control and fertilizer powder-treated microcosms appeared to be approximately the same. Cobble systems showed some rocks with clean surfaces, but there were generally fewer than in the oleophilic fertilizer-treated systems. Oil on the rock surfaces appeared gray and dried. Oil under the rocks was drier and less fluid than oil observed in the oleophilic fertilizer-treated microcosms.

After sampling the microcosms it was noted that the inside walls of the fertilizer powder-treated microcosms and the reference microcosms were spotted with oil smudges. This was not the case in the oleophilic fertilizer-treated set, where the walls generally appeared free of oil. Small particles of white waxy material were also observed throughout the sand and gravel in the oleophilic fertilizer-treated set of microcosms, even with the daily influx of fresh seawater. This material may have been residual oleophilic fertilizer, suggesting possible over application.

In a sand and gravel microcosm sampled 17 days post fertilizer application, the nC17/pristane and nC18/phytane ratios in the oleophilic fertilizer-treated microcosms were the same as those in the untreated microcosms (Table 6.28). Ratios for fertilizer powder-treated microcosms were almost half the ratios for the other microcosms, and there was also approximately 20% less oil residue by weight. These data suggest that the more rapid degradation of oil was occurring in the fertilizer powder treatments, assuming oil concentration and composition were approximately the same in all microcosms at the start of the experiment. Unfortunately, the t=0 samples were lost. However, since a single batch of homogenized beach material was used to construct the microcosms and the consistency between replicates was generally good, the effect of enhanced oil biodegradation seems reasonable. Because of the large amount of readily degradable carbon added with the oleophilic fertilizer, enhanced degradation of the oil may not occur until after much of this carbon is degraded.

TABLE 6.28. CHEMICAL ANALYSIS OF MIXED SAND AND GRAVEL MICROCOSMS
SAMPLED 17 DAYS AFTER INITIATION OF FERTILIZER APPLICATION

Treatment	Residue Weight (mg/kg)	nC17/Pristane	nC18/Phytane
Control 1	1,570	0.5	0.8
Control 2	913	0.4	0.6
Control 3	790	0.4	0.6
Average	1,091	0.4	0.7
Oleophilic 1	1,490	0.4	0.7
Oleophilic 2	1,360	0.4	0.8
Oleophilic 3	795	0.4	0.7
Average	1,215	0.4	0.7
Fert. powder 1	913	0.3	0.4
Fert. powder 2	916	0.1	0.3
Fert. powder 3	845	0.3	0.3
Average	891	0.2	0.3

In the cobble microcosms sampled 26 days post application of fertilizer, similar results were observed. The lowest oil residue weights (Table 6.29) and the greatest change in composition (as measured by the nC17/pristane and nC18/phytane ratios in Table 6.30), appeared to be in the fertilizer powder-treated systems. Oleophilic fertilizer-treated and untreated systems were approximately the same. The results were relatively consistent between replicates and with different layers in the microcosm. Thus, it is again tempting to assume that the lower residue weights and ratios were due to fertilizer-enhanced biodegradation. Oil residue weights in the oleophilic fertilizer-treated systems were as high as 6 times those in the control microcosms. This indicates that a component of the oleophilic fertilizer was possibly contributing to the residue weight and that over application had occurred.

In contrast to day 26 data, nC17/pristane and nC18/phytane ratios for the day 41 samples suggest that oil was being degraded faster in the control microcosms than it was in the fertilizer powder-treated microcosms (Table 6.31). However, the hydrocarbon ratios may yield false indications of biodegradation if pristane or phytane are degraded along with straight chain hydrocarbons. Gas chromatography/mass spectrometry analysis of the data provided sufficient data to evaluate this possibility (Table 6.31). By extracting and analyzing all microcosm samples using the same method, two compounds whose concentrations did not change in any of the treatments were identified: norhopane and hopane. The ratio of norhopane to hopane remained constant at 0.76 (Table 6.31). Constructing nC17/norhopane and pristane/norhopane ratios indicated that nC17 degradation was 5 times more in the fertilizer powder-treated microcosms than in the control microcosms (Table 6.31). Based on the same ratio method, pristane was also degraded in both the control and fertilizer powder-treated microcosms, supporting the suggestion that nC17/pristane ratios could be misleading.

In addition, the ratios of the three major dibenzothiophene peaks to norhopane were also examined using mass spectral analysis (Table 6.32). Further differences between the treatments were apparent. Fertilizer powder-treated microcosm samples showed the lowest ratios. Interestingly, the ratios for the oleophilic treatment indicated little change in the dibenzothiophene isomers, compared with the ratios observed in a Prudhoe Bay crude oil standard. These observations are consistent with the nC17/pristane data from previous samplings, which also indicated that oil degradation in the oleophilic treatment was less than the degradation in both the fertilizer powder and control treatments.

TABLE 6.29. RESIDUE WEIGHT OF OIL IN COBBLE MICROCOSMS ANALYZED 26 DAYS
AFTER FERTILIZER APPLICATION

Treatment	Top Cobble	Bottom Cobble	Gravel
		Residue Weights (mg/kg)	
Control 1	1,420	1,120	889
Control 2	889	1,090	1,090
Control 3	1,040	722	1,030
Average	1,116	977	1,993
Oleophilic 1	1,770	1,910	6,350
Oleophilic 2	1,260	2,460	5,580
Oleophilic 3	2,340	3,550	6,960
Average	1,790	1,640	6,297
Fert. powder 1	161	1,310	1,020
Fert. powder 2	1,240	725	714
Fert. powder 3	383	664	814
Average	595	900	849

TABLE 6.30. RATIOS OF HYDROCARBONS IN OIL FROM COBBLE MICROCOSMS
ANALYZED 26 DAYS AFTER FERTILIZER APPLICATION

Treatment	nC17/Pristane			nC18/Pristane		
	Top Cobble	Bottom Cobble	Gravel	Top Cobble	Bottom Cobble	Gravel
Control 1	0.8	0.7	0.3	1.3	1.1	0.5
Control 2	0.9	0.6	0.4	1.3	1.0	0.5
Control 3	0.7	0.7	0.4	1.3	1.1	0.5
Average	0.8	0.7	0.4	1.3	1.0	0.5
Oleophilic 1	0.9	0.8	1.0	1.3	1.4	1.5
Oleophilic 2	1.0	0.9	0.9	1.2	1.5	1.4
Oleophilic 3	1.0	0.9	0.8	1.2	1.4	1.3
Average	1.0	0.9	0.9	1.2	1.4	1.4
Fert. powder 1	0.2	0.3	0.5	0.4	0.4	0.5
Fert. powder 2	0.6	0.1	0.6	1.1	0.3	0.7
Fert. powder 3	0.6	0.2	0.5	0.9	0.3	0.5
Average	0.5	0.2	0.5	0.8	0.3	0.5

TABLE 6.31. COMPARISON OF NC17/PRISTANE RATIOS AND NC17/NORHOPANE RATIOS
AS MEASURES OF OIL DEGRADATION IN SAMPLES TAKEN FROM COBBLE MICROCOSM
42 DAYS AFTER INITIATION OF FERTILIZER APPLICATION

Microcosm	nC17/ Pristane	nC17/ Norhopane	Pristane/ Norhopane	Norhopane/ Hopane
Control	0.19	1.03	5.44	0.78
Fert. powder	0.49	0.22	0.44	0.75
Fresh Prudhoe Bay Crude Oil	1.7	17.50	10.68	0.78

TABLE 6.32. USE OF DIBENZOTHIOPHENE PEAKS/NORHOPANE RATIOS
AS RELATIVE MEASURES OF THE DEGRADATION OF AROMATIC COMPONENTS
IN OIL SAMPLED FROM COBBLE MICROCOSMS 42 DAYS AFTER INITIATION
OF FERTILIZER APPLICATION

| Microcosms[b] | Dibenzothiophene Peaks[a]/Norhopane Ratios | | |
	Peak 1	Peak 2	Peak 3
Control 1	.40	.54	.60
Control 2	.49	.66	.71
Control 3	.46	.70	.71
Fert. powder 1	.08	.13	.13
Fert. powder 2	.10	.12	.19
Fert. powder 3	.11	.13	.17
Oleophilic 1	.82	1.21	1.06
Oleophilic 2	.81	1.15	1.01
Oleophilic 3	.85	1.17	.99
Fresh Prudhoe Bay Crude Oil	1.06	1.84	1.54

[a] In the mass spectral analysis of oil, C-2 dibenzothiophenes and their homologs show a series of peaks at mass ion 212. Three prominent peaks (labeled here 1, 2, and 3) were selected for comparison.
[b] Average of three replicates.

From these initial microcosm results, it can be concluded that enhanced oil biodegradation will occur if sufficient nutrients are supplied to the microorganisms. Because microcosms represent test systems that best reflect field conditions, a similar response could be expected in the field if nutrient concentrations were maintained at adequate levels. The lack of any effect of the oleophilic fertilizer was probably due to over application. The microcosm studies also showed that pristane and phytane are biodegraded and therefore must be used with caution when assessing changes in oil composition.

Jar Microcosms

The results from these studies indicated that the addition of oleophilic fertilizer led to a substantial increase in the number of organisms capable of growth on oleic acid-agar plates (Figure 6.91). High background concentrations of oleic acid-degrading bacteria were observed in the water even before oleophilic fertilizer treatment.

Since the aqueous phase at each water change was sterilized, the number of oleic acid-degraders may reflect those that sloughed off the oiled rocks during a 24-hour period. However, no obvious differences were observed for the different aqueous phases. Similar results were observed in systems that did not have daily water changes.

Results from the enumeration of oil-degrading organisms indicated that in all cases the populations increased to a high value by Day 3 and then decreased to an intermediate but variable level for the following 6 days (Figure 6.92). Similar results were seen in those jars that did not have a daily water change. Although all samples showed a peak after 3 days of incubation, jars containing only seawater appeared to have the fewest microorganisms in the 6 days following the 3-day peak. Chemical analysis of the water samples is being performed. Information on how effectively the enriched oleic acid-degraders can degrade the oil also is forthcoming.

Oleophilic fertilizer increased the number of oleic acid-degrading bacteria in flask studies designed to approximate field conditions. This situation would theoretically result in competition for available nutrients between oleic acid-degrading and oil-degrading bacteria. This competition could explain the decrease in oil-degrading bacteria following their initial rise after initiation of the experiment. Supplying dissolved nutrients in addition to nutrients in the oleophilic fertilizer did not seem to affect the oleic acid- and oil-degrading bacterial populations.

Figure 6.91. Effect of INIPOL on the Relative Numbers of Oleic Acid-Degrading Microorganisms in Jars Containing Oiled Rocks and Artificial Seawater, Seawater, or Saline Solution. Incubated with Daily Change of Water.
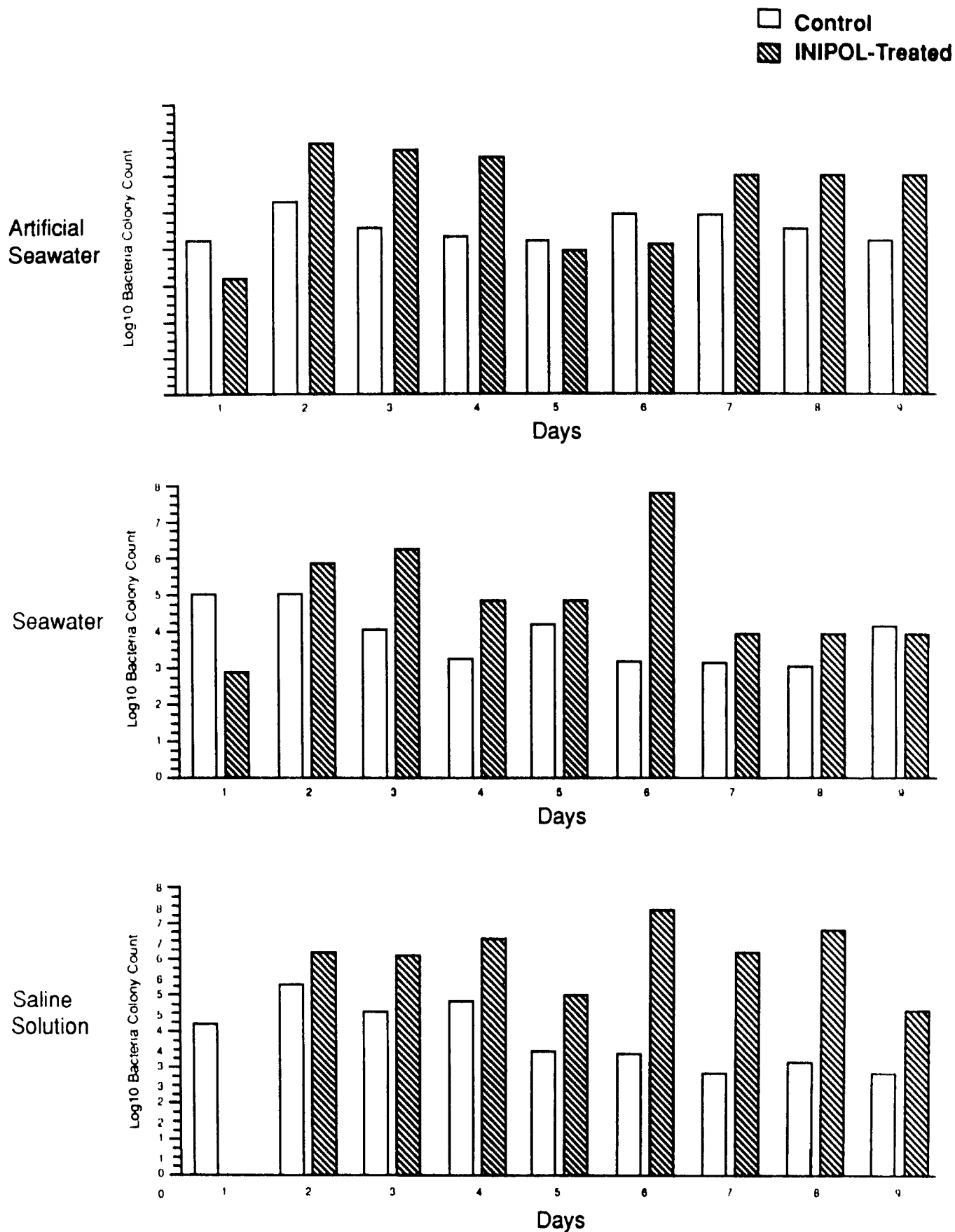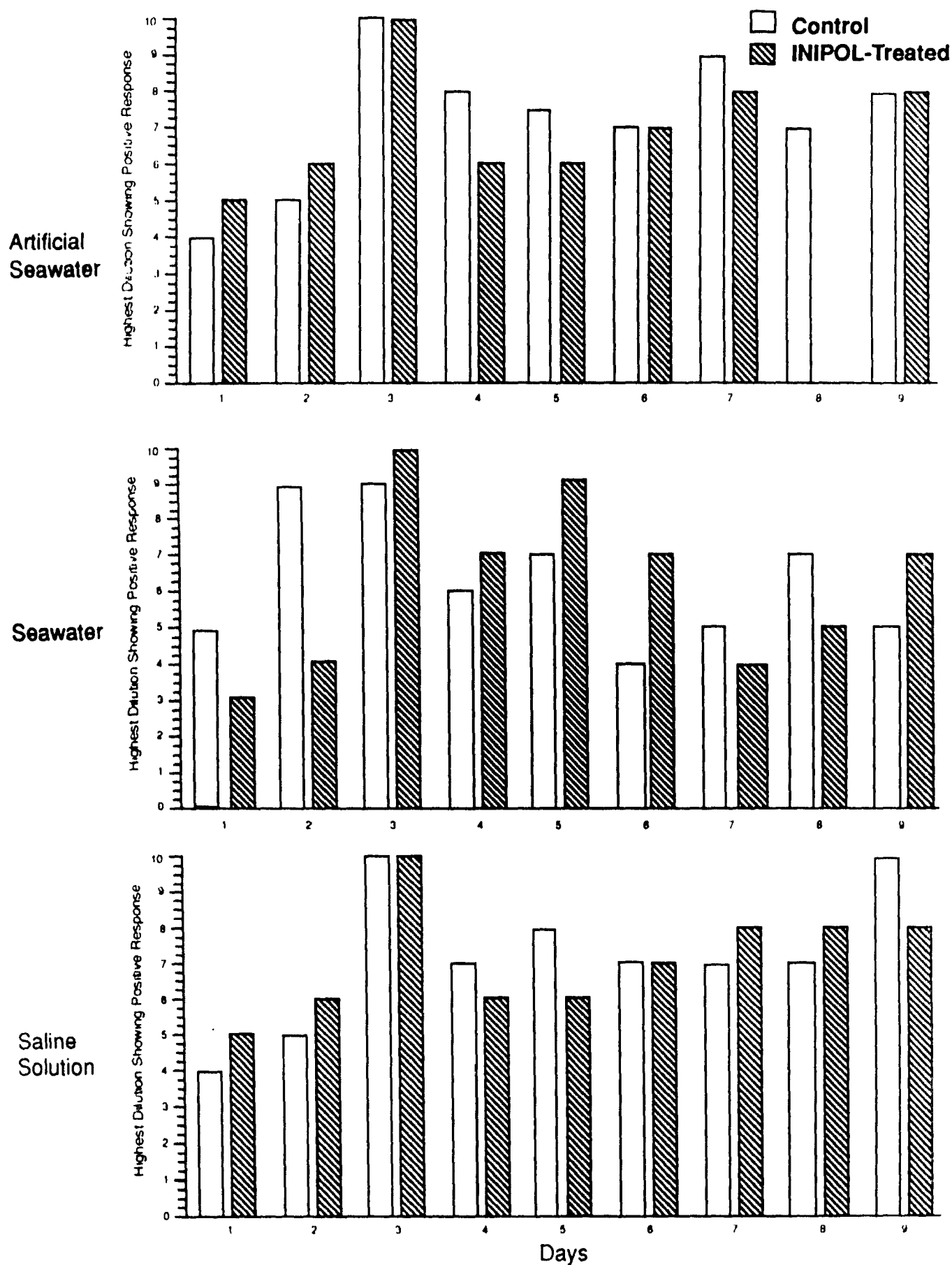
Figure 6.92. Effect of INIPOL on the Relative Numbers of Oil-Degrading Microorganisms in Jars Containing Oiled Rocks and Artificial Seawater, Seawater, or Saline Solution. Incubated with Daily Change of Water.

MUTAGENICITY TESTS

Experiments were initiated at Snug Harbor to determine the potential mutagenic activity associated with biodegradation of oil. The types of health hazards for which monitoring is most difficult are those with chronic, delayed effects such as carcinogenicity, neurotoxicity, and mutagenicity. Fortunately, for mutagenicity there are short-term *in vitro* tests that demonstrate whether or not a pollutant interacts in a detrimental manner with DNA. Due to the mechanistic research with oncogenes, available evidence shows that oncogene activity can be initiated by mutation. Mutation assays, although not definitive, can be used as screening tests for the presence of potential carcinogens. When performed in a quantitative, dose-responsive fashion, these bioassays can be used to detect alterations in the quantity of mutagens present within complex mixture samples.

Potential health effects from the oil spill were assessed by examining the mutagenicity associated with the oil spill, the weathered oil, and the products associated with bioremediation. The most commonly used mutation assay is the *Salmonella typhimurium*/mammalian microsome assay developed by Ames. An early pilot study had demonstrated that extracts of spilled oil were mutagenic in the *Salmonella typhimurium* bioassay for mutagenicity. This meant that the removal of genotoxic components from the oil by biodegradation could be monitored with this assay.

Both the Prudhoe Bay crude oil and the weathered oils tested were weakly mutagenic using TA100. The commercial fertilizer formulations were non-mutagenic. Figure 6.93 shows the mutagenicity of oil samples collected from an untreated control site, an oleophilic fertilizer-treated site, and a water-soluble fertilizer-treated site within sandy/gravel and cobblestone beach areas. Examination of the mutagenicity per amount of soil (Figure 6.93) shows that the overall mutagenic activity of the gravel beach is higher than the cobblestone beach. This activity tends to persist for the four month period. On both types of beaches, however, the mutagenicity declines with time. It is interesting to note that the decline was observed for both the untreated control and treated beaches. By the time the first samples were collected in June, natural processes may have already initiated natural bioremediation activities (e.g., the snow melt on the mountain may have washed organic matter into the beach area). In addition, the fresh water outflow and wave action may have removed oil spill organics from the beach.

The chemical analyses, however, did not determine whether or not mutagens were removed from the soil at a rate commensurate with other residue chemicals. Figure 6.93B shows that the

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Revertants per gram soil

1,000
500
200
100
50
20
10
5

291 141 184 284 126 838 | 504 39 NA 137 NEGATIVE NEGATIVE | 34 NEGATIVE NEGATIVE 87 255 27 | 431 6 134 36 119 208 | 12 NA 66 366 122 116 | NEGATIVE NEGATIVE NEGATIVE NEGATIVE 41 36 NEGATIVE

A

1 2 1 2 1 2 | 1 2 1 2 1 2 | 1 2 1 2 1 2 | 1 2 1 2 1 2 | 1 2 1 2 1 2 | 1 2 1 2 1 2

REF OLEO W-S | REF OLEO W-S | REF OLEO W-S | REF OLEO W-S | REF OLEO W-S | REF OLEO W-S

June   July   Sept.   June   July   Sept.
Gravel Beach   Cobblestone Beach

Legend

Ref = Untreated Control
Oleo = Oleophilic Fertilizer
W-S = Water-Soluble Fertilizer

Rev / mg Extract

100
80
60
40
20
0

75.5 70.3 67 | 50.5 45.5 NEGATIVE 4.3 | 49.5 45 | 58.3 51.5 55.8 | 21 95 25 | NEGATIVE 22.8 35

B

% Extractable

1
0.1
0.01
0.001

C

REF OLEO W-S | REF OLEO W-S | REF OLEO W-S | REF OLEO W-S | REF OLEO W-S | REF OLEO W-S

June   July   Sept.   June   July   Sept.
Gravel Beach   Cobblestone Beach

Figure 6.93. Mutagenicity of Soil Extracts Using the Spiral *Salmonella typhimurium* Assay (Houk et al., In Press) with Strain TA98 with an Aroclor 1254-Induced CD-1 Rat Liver Homogenate (Atlas and Pramer, 1990) Exogenous Activation System. The Spiral Assay Analysis Provided a Net Increase in Revertant Colonies per Amount of Substance Tested. (A) Mutagenicity Per Gram of Soil for Samples Taken From Sandy/Gravel and Cobblestone Beaches. Each Beach Type was Represented by an Untreated Control Site (No Fertilizer); Oleo (Oleophilic Fertilizer); and W-S Site (Water-Soluble Fertilizer). Numerals 1 and 2 for Each Treatment Indicate the Two Randomly Selected Grids Used for Sampling. Each Sample was Bioassayed Twice and the Means are Presented. (B) Mutagenicity of the Extracted Organic Material Expressed as Revertants per mg Organic Material. The Values Represent the Means of Samples Taken from Two Representative Grids with Each Sample Tested Twice. (C) The Mean Percent of Extractable Mass for the Samples Represented In Graph B. Samples Were Extracted with Dichloromethane.

mutagenicity per mg of extractable matter (oil residue) decreased with time. Since this is not expressed on a total volume or weight of soil basis, it demonstrates that the mutagenic components were being depleted at a faster rate than the overall organic content. Figures 6.93B and 6.93C also show that both the mutagenic activity per amount of extractable organic matter and the percent of extractable mass decreased over time.

These mutagenicity studies show that mutagenic toxins associated with spills of Prudhoe Bay crude oil were lost over time. In conjunction with chemical analysis, these studies demonstrated that decreases in toxicity were due to both fertilizer-enhanced bioremediation and other natural processes. In addition to ongoing laboratory studies that are examining the mutagenicity of other oils and their degradation products, these studies will assist in selecting appropriate bioremediation procedures for environmental oil spills.

SUMMARY AND CONCLUSIONS

Based on analysis of the data from the bioremediation field demonstration in Snug Harbor in the summer of 1989 the following general discussion and conclusions can be drawn:

a) Visual inspection of beaches treated with oleophilic fertilizer showed that oil was removed from the beach surface approximately 2 to 3 weeks after fertilizer application. The effect was most apparent on cobble beaches, where initially much of the surface oil was removed. No visible decreases in the oil occurred, however, on the beaches treated with the slow-release fertilizer briquettes or on the untreated control beaches. Disappearance of oil on oleophilic-treated plots continued over time, eventually leading to the disappearance of oil from most of the beach material surfaces.

b) No oil slicks or oily materials were observed in the seawater following application of the fertilizers, and no oil or petroleum hydrocarbons were detected in mussels contained in cages just offshore from the fertilizer-treated beaches. This suggested that removal of oil from the beaches did not appear to be a result of dispersing phenomena.

c) Analysis of oil extracted from all beach plots showed that the effect of fertilizer application on the loss of oil residues from the test plots was only apparent in oil samples taken from the cobble surface of the INIPOL fertilizer-treated plots. Results were statistically significant

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at the 95% confidence interval. The enhancement effect, however, lasted for only four weeks, suggesting that the supply of nitrogen and phosphorus from the fertilizer was depleted during this period. This oil disappearance corresponded to both visual observations and the detection of elevated nutrient concentrations on this plot. Although it is tempting to attribute this loss to biodegradation, further analysis of changes in oil composition must be conducted to substantiate the potential role of biodegradation. None of the other types of beach samples (oil from mixed sand and gravel under cobble and oil from mixed sand and gravel alone) or fertilizer treatments showed enhanced oil removal relative to the untreated control. The absence of any effect on the oil in the mixed sand and gravel under cobble may have been due to very low initial oil concentrations (little room to see changes) and highly erratic distribution in the beach material. If we entertain the possibility of a prominent role of biodegradation in determining oil fate, it is quite likely in the mixed sand and gravel plots that not enough nutrients were delivered to oil-degrading microbial communities in the beach material to promote a sufficient enhancement of oil degradation and, thus, cause a significant decrease in oil concentration. On the other hand, oil-degrading microbial communities within the beach material may not have had time to become as enriched as those on the surface of the cobblestone. Thus, we would suggest that INIPOL works best on surface oil and that additional fertilizer should be added to supply nutrients to developing microbial communities in the subsurface.

d) Due to the very heterogeneous distribution of oil on the beaches, imprecise methods for sampling unconfined gravel and cobble, and high amounts of natural oil biodegradation, it was not possible to statistically link visual changes with enhanced removal of oil residues. However, trends in the data strongly suggest that the most substantial loss of oil residue occurred in the cobble surface samples taken from the oleophilic fertilizer-treated beaches, particularly during the first 20 to 30 days of the test. During the latter part of the test period, oil residue losses on all beaches seemed to dramatically increase beginning sometime in the middle of July, leading to a virtual cleaning of all test beaches. The factors responsible for this increase are not known.

e) Samples of oil from fertilizer-treated beaches, particularly from cobble surfaces, that were taken around the time the oil was visually disappearing showed substantial changes in hydrocarbon composition. This indicated extensive biodegradation, and suggested that biodegradation might also be affecting oil removal, both through direct decomposition and

possibly through the production of biochemical products (bioemulsifiers) known to be produced by bacteria as they consume oil and hydrocarbons as food sources. Changes in oil composition, including the standard measure of oil biodegradation involving the ratio of specific branched alkanes to rapidly degraded straight-chain alkanes, were greatest on the beach treated with fertilizer briquettes. The effect was most pronounced during the first 20 to 30 days of the test in cobble plot samples, suggesting that the fertilizer-enhanced changes were short-lived. Depending on the hydrocarbons measured, the application of oleophilic fertilizer also significantly enhanced changes in oil composition, but to a lesser extent. It is hypothesized that the presumed ability of the oleophilic fertilizer to hold nutrients within the oil-degrading microbial communities led to a greater mass of oil degraded. This degradation included mineralization to CO_2 and conversion into microbial biomass. This in turn changed the physical consistency of the oil, thereby allowing the degraded oil to be sloughed off the beach material by tidal action. Thus, oil biodegradation in Prince William Sound was nutrient limited and rates were enhanced by the addition of fertilizer. These results also lead to the conclusion that fertilizer briquettes, or a similar formulation that releases inorganic nitrogen and phosphorus, would likely affect changes in oil composition on both the cobble surface and within the mixed sand and gravel matrix.

f) All changes in oil composition were accompanied by large decreases in the nC18/phytane ratio. This represents a differential change in chemically similar hydrocarbons, and can only be attributed to biodegradation processes. Thus, fertilizer application appeared to enhance oil biodegradation.

g) Numbers of oil-degrading microorganisms did not appear to increase as a result of fertilizer application. However, large heterogeneity in the microbial population precluded observing statistical differences. This was further complicated by the awareness that the numbers of oil-degrading bacteria in the oiled beach material before exposure to fertilizers were very high, averaging 1 to 10% of the total bacterial population. The high numbers represented an enrichment of oil-degrading microorganisms of approximately 10^3 to 10^5 as compared to beach microorganisms not exposed to oil. These results demonstrate that the beaches were well primed for bioremediation.

h) Extensive monitoring studies indicated that the addition of fertilizer to oiled shorelines did not cause ecologically significant increases in planktonic algae or bacteria, or any measurable

nutrient enrichment in adjacent embayments. In addition, mutagenicity studies showed that mutagenic materials associated with Prudhoe Bay crude oil were lost over time from both treated and untreated control plots. In conjunction with chemical analysis, these studies demonstrated that decreases in mutagenicity were due to both fertilizer-enhanced biodegradation and other natural processes.